## Remarks

After amendment, newly added claims 39-73 remain pending in the present application. Applicant submits this amendment in order to place the application in condition of allowance and to expedite allowance of the instant application. The amendments made herein are made for purposes of clarifying the invention. Applicant respectfully submits that the instant claims which are directed generally to methods of treatment which make use of the disclosed compounds' unexpected activity as it relates to their Selective Estrogen Receptor Modulator (SERM) activity are patentable over the art of record.

Applicant respectfully submits that the presently pending claims are patentable over the prior art cited of record, inasmuch as that art is relied upon by the Examiner for the teaching of *exclusively estrogenic activity* of the disclosed compounds, not SERM activity as in the present method. Moreover, the method claims which are presented, specifically treat disease states and/or conditions where the compounds' SERM activity provides activity which is clearly distinguishable over the cited art. In the presently claimed methods, the putative estrogenic activity of the prior art compounds are *contraindicated* for use in the presently claimed methods. Contrary to the Examiner's position, there is simply no teaching or motivation of the methods of the present invention which rely on the unexpected SERM activity. Thus, according to the methods of the present invention, SERM compounds may be used to treat estrogen-sensitive cancers (including breast cancer), to reduce the likelihood of the occurrence or recurrence of estrogen-sensitive cancers in patients at risk for those cancers and to treat menopausal symptoms which are specifically claimed, without increasing the risk of or exacerbating estrogen-sensitive cancers in the treated patient. The prior art neither discloses nor suggests the methods of the present invention inasmuch as the teachings there involve the putative exclusive estrogenic activity of the disclosed compounds. It is respectfully submitted that compounds of the prior art which are presumed (incorrectly) to exhibit exclusively estrogenic activity would not be understood to be useful in the methods of the present invention which are contraindicated to the prior art teachings. Indeed it is the unexpected activity of the present compounds as *SERMS*

which provides the basis of the invention of the present application.

It is noted that because the compounds which are used in the present invention exhibit selective estrogenic activity (i.e., agonist activity at certain receptors and antagonist activity at other receptors), the methods of the present invention are reflective of that selective estrogenic activity. Thus, in the present invention, recognition of this unexpected SERM activity gives rise to a method for treating estrogen-sensitive cancers, a method for treating menopausal symptoms as claimed in a patient who also is stricken with an estrogen-sensitive cancer and the other methods as claimed. The claims are clearly distinguishable over the art of record.

It is clear that the prior art does not disclose these methods and it is also clear that the prior art, which teaches the exclusive (desirable) estrogenic activity of the disclosed compounds does not suggest or motivate one of ordinary skill in the art to practice the presently claimed methods. Indeed, if anything, the prior art *teaches away* from the present invention inasmuch as compounds which are disclosed in the prior art as exhibiting *exclusively* estrogenic activity would not be used in the methods of the present application which are directed to the unexpected SERM activity of compounds which are claimed in the present methods. Indeed, from the art of record, there was no suggestion or even an oblique reference to the invention of the present application, nor was there motivation to practice the present method from the prior art disclosure.

The Examiner has rejected newly submitted claims 39-73 variously under 35 U.S.C. §112, first and second paragraphs, and 35 U.S.C. §103 for the reasons which are stated in the office action on pages 2-9. Applicant respectfully traverses the Examiner's rejections in the sections which are presented infra.

**The Rejections Under 35 U.S.C. §112, first and second paragraphs**

*The Rejection of Claims 39-47 and 65-73 Under 35 U.S.C. §112, First Paragraph As Failing to Comply with the Written Description Requirement*

The Examiner has rejected claims 39-47 and 65-73 under 35 US.C.§112, first paragraph as failing to comply with the written description requirement for the reasons which are presented in the office action on pages 2-3. Essentially, it is the Examiner's position that the term "treating the symptomology of menopause" which is recited in independent claims 39 and 65 as amended now meets the requirements of 35 U.S.C. §112, first paragraph as it relates to the written description requirement. Note that Applicant has amended claims 39 and 65 to reflect the specific symptomology which is treated by the present methods, in particular, bone loss associated with osteoporosis, elevated cholesterol, elevated low-density lipoproteins (LDL) and cardiovascular disease.

It is noted that the use of the present compounds which exhibit activity as SERMS are useful for treating those aspects of menopause which are specifically enumerated in the specification. See the specification at page 5, second full paragraph. The compounds are also useful for the treatment of estrogen-sensitive cancer as well as reducing the likelihood of the occurrence or recurrence of estrogen-sensitive breast cancer. Those aspects of the invention are taught throughout the originally filed specification, and in the objects of the invention on pages 2-3, as well on page 5, in the first and second full paragraphs, page 7, second, third and fourth full paragraphs, as well as the fifth full paragraph on page 7, page 8, third paragraph, lines 2-5. As indicated, the specification is *replete* with support for the treatment of the symptomology of menopause as well as reducing the likelihood of a patient contracting an estrogen-sensitive cancer. It is respectfully submitted that the present application and in particular, amended claims 39-47 and 65-73 completely satisfy the requirements of 35 U.S.C.§112, first paragraph as those claims relate to the written description requirement.

*The Rejection of Claims 39-47 and 65-73 Under 35 U.S.C. §112, First Paragraph As Failing to Comply with the Enablement Requirement*

The Examiner has rejected claims 39-47 and 57-64 under 35 U.S.C.§112, first paragraph as failing to comply with the enablement requirement for the reasons which are set forth in the

office action on pages 4-5. In particular, the Examiner contends that, in order to practice the invention of claims 39-47 and 57-65, one of ordinary skill would have to identify "a patient" at risk for developing estrogen-sensitive cancer or with the likelihood of a recurrence of breast cancer. The Examiner contends that one of ordinary skill in the art would be unable to identify an individual "at risk for developing estrogen-sensitive cancer" or in need of "reducing the likelihood of a recurrence of breast cancer" and that such identification would represent undue experimentation in practicing the present invention. Applicant respectfully traverses the Examiner's rejection.

The invention of claims 39-47 and 57-64 does require that one of ordinary skill be able to identify a patient who is "at risk for developing an estrogen-sensitive cancer" (claim 39) or "at risk for a recurrence of breast cancer", however identifying these patients is within the routineer's skill in the art. For example, women who consume alcohol, especially reasonably heavy drinkers are at risk for estrogen-senstive cancers and women who enter menopause later in life (generally, after age 55) are also at risk for estrogen-sensitive cancers. Also, women who have taken estrogen plus progesterone hormone therapy or who have not had children or breastfed are also at higher risk for estrogen-sensitive cancer. In addition, a patient's diet has significant implications for risk of estrogen-sensitive cancer as does a patient's smoking, and diets high in soy isoflavones and certain types of fish (especially catfish) increase the risks of an occurrence of estrogen-sensitive cancer. Environmental exposures also significantly increase the risk of breast cancer with a patient's exposure to xenoestrogens such as nonylphenol (somewhat ubiquitous in personal care products), bisphenol, DDT, methoxychlor, aromatic hydrocarbons, and pesticides increasing the likelihood of estrogen-sensitive cancer. In addition, measuring blood levels of estrogen (high levels pose a greater risk for cancer) or determine the ratio of estrogen's hydroxylated metabolites 2-hydroxyestrone and 16-hydroxyestrone, as well as taking a biopsy of tissue and determining that precancerous cells are present in relevant tissue of the patient provide further indication and assessment of the risk of estrogen-sensitive cancers. In short, there are numerous indicators of higher risk for estrogen-sensitive cancers which can be determined for a patient and one of ordinary skill in the art may readily make use of such indicators in assessing

the risk of an estrogen-sensitive cancer occurring in a patient. In the event of such a determination, the administration of compounds according to the present invention to treat the menopause symptoms as claimed may be readily accomplished simply by following routine practices in the art and the teachings of the present invention in the present application.

Turning to the question of assessing the risk of a recurrence of an estrogen-sensititive cancer (claim 57), it is respectfully submitted that such a determination is readily made by the routineer, generally, an oncologist who has already treated a patient for an estrogen-sensitive cancer which resulted in remission. It is noted that the population at risk for a recurrence of an estrogen-sensitive cancer, has similar risks to those which are described above for those at risk for estrogen-sensitive receptors, but also includes risk factors which are generally associated with the severity of the cancer originally treated. Typically, the approach for assessing such a risk involves diet and other factors as described above, including the analysis of estrogen levels, estrogen metabolites, and the existence of precancerous tissue by biopsy. A patient that is at risk for an occurrence or recurrence of an estrogen-sensitive cancer and who has menopausal symptoms such as osteoporosis, elevated levels of cholesterol, low-density lipoproteins and an elevated risk of cardiovascular disease as claimed are treated with compounds according to the present invention which exhibit excellent activity in treating the menopausal symptoms, but which do not exacerbate or increase the risk of an occurrence or recurrence of an estrogen-sensitive cancer because the present compounds exhibit estrogen inhibition within the context of the etiology and development of estrogen-sensitive cancers. Thus, in contrast to estrogen agonists, the compounds of the present invention, which are selective estrogen receptor modulators, allow for the treatment of the menopause symptoms as claimed without exacerbating the risk of cancer, in contrast to estrogen agonist, which do not exhibit the selective activity of the present compounds.

Turning to the question of exemplification/enablement of the present methods, it is respectfully submitted that the examples which are taught in the specification on pages 21-25 provide sufficient evidence of the general activity of the present compounds as SERMS. That

activity presented in the specification, coupled with the clinical activity of the nonstearoidal SERMS such as tamoxiphen, raloxifene, etc. which are currently used clinically, evidences the utility and enablement of the instant claims. See, for example, C. McNeil, *J. Nat. Can. Inst.*, 1998, 90(13):956-957; Jordan and Morrow, *Endocrine Reviews,1999,*20(3), 253-278; V.C. Jordan, *J. Cell. Biochem. Supp.*1995, 22, 51-57; and Olevsky and Martino, *Menopause*, 15, pp. 790-796 2008, copies enclosed for the Examiner's review.

Based upon the foregoing, it is respectfully submitted that the claims are now in compliance with the requirements of 35 U.S.C. §112, first paragraph.

*The Rejection of Claims 39-73 Under 35 U.S.C. §112, Second Paragraph As Being Indefinite*

The Examiner has rejected claims 39-73 under 35 U.S.C. §112, second paragraph as being indefinite for the reasons which are stated in the office action on pages 5-6. In particular, the Examiner rejects previously pending claims 39, 48, 57 and 65 for using the term "preferably", which has been deleted from those claims and rejects previously pending claims 39 and 48 for having X substituents bonded to improper positions in the moiety, which has been corrected.

Separately, claims 39, 48 and 65 recite the term "estrogen-sensitive cancer", which, according to the United States National Cancer Institute (see, http://www.cancer.gov/cancertopics/aromatase-inhibitors), refers to any cancer which is helped in its growth by exposure to estrogen. Examples of estrogen-sensitive cancers include breast cancer, estrogen-sensitive ovarian cancer and estrogen-sensitive uterine (endometrial) cancer. It is respectfully submitted that the term "estrogen-sensitive cancer" as used by those of skill in the art is definite, refers to the indicated cancer which one of ordinary skill in the art will readily recognize. It is respectfully submitted that the term as used in claims 39, 48 and 65 requires no further presentation or elaboration to make the claims definite.

It is respectfully submitted that claims 39-73 are now in compliance with the

requirements of 35 U.S.C. §112, first and second paragraphs.

**The Rejections Under 35 U.S.C. §103**

The Examiner has rejected claims 39-56 and 65-73 under 35 U.S.C. §103 as being unpatentable over Van den Broek, et al., US. Patent no. 3, 972,906 ("the '906 patent) for the reasons which are spelled out in the office action on pages 7-8. Essentially, it is the Examiner's position that the '906 patent teaches the use of compounds exhibiting estrogenic activity which is useful for the treatment of estrogen-deficiency syndromes. The Examiner cites several references disclosing chemical structures unrelated to the presently claimed compounds as supporting the view that estrogenic compounds are useful in treating breast cancer. From these references, the Examiner concludes that it would be prima facie obvious to use the compounds as disclosed in the '906 patent, in the methods of the present application. Applicant respectfully traverses the Examiner's rejection.

It is the Examiner's view that because the '906 patent generically discloses a number of compounds which are said to be potentially useful in the treatment of estrogen-deficiency syndromes, that disclosure suggests the present claims. Applicants respectfully strongly disagree.

*The '906 Patent Does Not Teach The Unique SERM Activity of the Presently Claimed Compounds*

Applicant previously enclosed a copy of Jelínková, et al., *Acta Endocrinologica*, 96, 389-393 (1981) ("Jelinkova"), which shows the traditional testing of compounds for estrogenic activity (uterotropic and/or vaginal activity). This reference clearly supports Applicant's contention that the present compounds and methods were not known from the '906 patent and could not have been known from the '906 patent, because the present compounds are ***inactive*** in the traditional estrogenic assays of Jelinkova which tested uterotopic and/or vaginal activity of compounds thought to have *estrogenic* activity. According to the '906 patent and Jelinkova, in

order for a compound to be useful, that compound must test favorably in an assay which tested for uterotropic and/or vaginal) estrogenic activity. However, the present compounds do not evidence activity in the assay of Jelinkova, which was the means of testing for the activity of compounds of the '906 patent. Indeed, it the very activity which is viewed by the '906 patent as being inactive/undesirable and useless, which forms the very basis of the patentability of the presently claimed methods.

The '906 patent does not teach and cannot possibly motivate the presently claimed compounds and their use in the claimed methods because the present methods rely on the unexpected (and, according to the '906 patent ***undesirable***) activity of these compounds as selective estrogen receptor modulators (SERMS) having mixed inhibitory and agonist estrogen activity. In contrast, the '906 patent teaches and requires exclusive estrogenic agonist activity, which stands in complete contrast to the present invention, which requires selective anti-estrogenic activity. This is because the presently claimed compounds/methods do not exhibit or rely on the traditional uterotropic and/or vaginal estrogenic activity ***required*** by the '906 patent and in fact, rely on activity which is, in part, anti-estrogenic. The presently claimed methods make particular use of the unexpected activity of these compounds.

This was pointed out in the previously submitted declaration of Professor Richard Hochberg and is further evidenced by the previously submitted paper of Zhang, et al., *J. Med. Chem.*, 48, 1428-1447 (2005) ("the JMedChem article"), which was previously enclosed and referenced herein. In particular, in the JMedChem article, it was noted in figure 2a that a similar compound of Jelinkova, the methoxy methyl analog E2-1,1, was estrogenic but as the11β-sidechain was lengthened to the methoxy ethyl ether (E2-1,2), the compound is *much less estrogenic* and when lengthened again by 1 methylene unit to the methyl propoxy ether it is *inactive* – and instead becomes ***antiestrogenic***- as set forth in figure 2b. The attention of the Examiner was drawn to the fact that the same structure activity relationship holds in an uterotrophic assay (figure 5 of the previously submitted JMedChem article)- i.e., that as chain length increases at the 11-position, traditional estrogenic activity (as measured by the Jelinkova

assay) falls off and its antiestrogenic activity *increases*. It was previously submitted that this activity was completely unexpected and stands in complete contrast to the teachings of the '906 patent. Thus, the compounds relied upon by the presently claimed methods exhibit essentially no meaningful agonist estrogenic activity (they are antagonists) in those assays which establish activity of the compounds of the '906 patent. The only conclusion to be drawn from this analysis is that the '906 patent actually ***teaches away*** from the present invention.

The present invention thus relates to the unexpected properties of the claimed compounds and their use in the claimed methods which relies on those unexpected properties, which are methods which make use of the unexpected activity of SERM compounds which exhibit estrogenic activity at peripheral sites in a patient's body, such as bone, liver and blood vessels, thus making them useful for treating certain menopausal symptoms *as clalimed,* but which exhibit ***anti-estrogenic*** activity in other tissues such as the uterus, breast, vagina, thus making them useful for reducing the likelihood of the occurrence or recurrence of or treating estrogen-sensitive cancers. Thus, based upon this unique, unrecognized (by the '906 patent) activity, the present compounds and methods treat menopausal symptomology at the same time that estrogen-sensitive cancers may be treated and/or discouraged. The present compounds thus exhibit no uterotropic or vaginal activity, the traditional test of estrogenic activity and instead are ***anti-estrogens*** in those tissues. The present methods make specific use of this unique activity for the treatment of certain symptomology of menopause including osteoporosis, hypercholesterolemia or elevated level of LDL, cardiovascular disease and, for the treatment of estrogen-sensitive cancers, including breast cancer, precisely because of the compounds' unique anti-estrogenic activity. The present invention is neither disclosed nor suggested by the '906 patent.

The activity of the compounds according to the present invention as SERMS are completely novel and unique *vis-à-vis* the disclosure of the '906 patent, which did not disclose or suggest the unexpected activity of the instantly claimed compounds, because that activity is incompatible with the '906 patent teachings. Thus, the presently claimed methods were unrecognized by the '906 patent and the compounds of the '906 patent could not have been

recognized to be useful in the presently claimed methods because the disclosed activity in the '906 patent is completely inconsistent with their use in the present methods. The '906 patent *clearly* ***teaches away*** from the present invention, inasmuch as the activity taught by the '906 patent would teach one of ordinary skill the desirability of estrogenic agonist activity, the exact type of activity which would *increase* the risk of occurrence or recurrence or *exacerbate estrogen-sensitive cancer.* It is respectfully submitted that the present compounds and related methods as claimed clearly are patentable over the '906 patent.

The '906 patent is directed to a large number of steroidal compounds, only a small number of which are even related to the present invention. The broad spectrum of compounds disclosed generically by the '906 patent exhibit *inter alia,* contraceptive, estrogenic, progestational, ovulation-inhibiting, gonad-inhibiting and anabolic properties. Most of the compounds which are disclosed by the '906 patent are prophetic. Notwithstanding that deficiency, the '906 patent does not disclose or even remotely suggest that the compounds disclosed therein were even possibly useful to treat menopausal symptoms in a patient wherein estrogen-sensitive cancer was a risk. Certain compounds of the '906 patent are said to be useful as medicaments in the treatment of estrogen-deficiency-syndromes, but are not taught to be useful to treat estrogen-sensitive cancer. As a separate note, the references which the Examiner also cites as supporting the use of estrogenic compounds in the treatment of cancer do not even relate to the present compounds and in any event would be viewed as being at best, *useless* or worse, *contraindicated* in treating estrogen-sensitive cancer, which disease state is exacerbated by estrogens exhibiting agonist (estrogenic) activity.

Contrary to the Examiner's contention, the teachings of the '906 patent do not disclose or even remotely suggest the present invention. Compounds according to the present invention are important in that they exhibit mixed anti-estrogenic/estrogenic activity; estrogenic activity in those tissues which are relevant to the treatment of menopausal symptoms as claimed, and critically, ***anti-estrogenic activity*** in the uterus, breasts and vagina, thus being of particular relevance to the treatment of estrogen-sensitive cancers. As claimed, the present methods are

useful to treat conditions secondary to menopause *without* the side effects which often occur with estrogen treatment of menopause (increased risk or breast cancer and endometrial cancer). In fact, the present compounds, instead of causing estrogen-sensitive cancers, including breast cancer, are actually useful for *treating* (i.e. inhibiting growth) or discouraging (reducing the likelihood) or breast cancer, as claimed. This is activity which is completely unexpected from the teachings of the art. And it makes the compounds and methods particularly useful for treating menopausal symptoms in patients who are at risk for or have contracted estrogen-sensitive cancers. This is an activity which was completely missed by the '906 patent.

The present methods are therefore completely non-obvious in light of the teachings of the '906 patent. As mentioned, the '906 patent discloses a huge number of steroidal compounds having a multitude of uses and pharmacological effects. While the '906 patent does disclose certain steroidal compounds having general estrogenic (agonist) activity, there is no disclosure of the selective estrogenic/anti-estrogenic activity of compounds according to the present invention which are particularly useful in the presently claimed methods. Based upon the disclosure of the '906 patent, the present methods are non-obvious.

The '906 patent discloses certain compounds which possess estrogenic (agonist) activity. In general, estrogenic activity is assessed in various estrogen responsive models, e.g., estrogen receptor binding, endometrial growth, local vaginal activity and uterotrophic activity. In each of these models, the compounds of the '906 patent disclosure, to the extent they are labeled "estrogenic" would exhibit traditional estrogenic activity, as exemplified by Jelinkova, previously submitted. The present compounds, however, exhibit ***anti-estrogenic activity*** in the very assays which are typically used to assess estrogenic activity, thus making the present methods tenable because of the recognize by the present inventor of the unique unexpected activity which is neither taught nor even obliquely suggested by the '906 patent. Thus, the '906 patent did not disclose or suggest the unique activity of compounds according to the present invention which activity is particularly appropriate for use in the present methods which are neither disclosed nor even obliquely mentioned by the '906 patent.

In the first instance, the '906 patent teaches away from the very compounds which are used in the presently claimed methods. It is respectfully submitted that one of ordinary skill looking to synthesize a series of compounds having estrogenic (agonist) activity as disclosed by the '906 patent would be "taught away" or "led away" from the present invention inasmuch as the compounds found useful in the present methods would be seen to be inactive in any relevant assay testing for estrogenic activity (e.g., Jelinkova). Following a typical drug development protocol, one of ordinary skill would look to generate an initial structure activity relationship (SAR) determination which would teach the person of ordinary skill that compounds of the structure according to the present invention (where the $C_{11}$ position has a longer chain) would exhibit no relevant estrogenic activity for the purposes for which the compounds of the '906 patent were made. Thus, the person of ordinary skill following the teachings of the '906 patent actually would completely fail to recognize that the present compounds should even be made inasmuch as the activity/value of the compounds useful in the present methods would be unrecognized from the teachings of the '906 patent. This is confirmed by Jelinkova and the attached JMedChem article, previously presented.

None of the present compounds would show activity in any of the standard estrogen assays consistent with any useful activity as disclosed by the '906 patent and there is simply no teaching which suggests the compounds of the present invention should be made. And certainly the '906 patent, which does not even mention estrogen-sensitive cancer, does not teach or even obliquely suggest methods of treating estrogen-sensitive cancer, does not disclose or even remotely suggest the methods of the present invention. Indeed, the present methods are completely inconsistent with the '906 teachings regarding the desirability of estrogenic activity. Further, the '906 patent *motivates away* from the present compounds and claimed methods inasmuch as estrogenic activity in the standard assays is viewed as valuable, whereas the compounds used in the present invention are essentially inactive in those very assays. Moreover, the use of estrogenic activity of the '906 patent would be viewed as deleterious in treating estrogen-sensitive cancer as in the presently claimed methods, inasmuch as agonist estrogenic activity actually ***exacerbates*** the cancer, rather than treating it. Thus, it is respectfully submitted

that one of ordinary skill would understand from the '906 patent (as confirmed by Jelinkova and the JMedChem article) that the compounds used in the present methods were of no biological significance related to the '906 teachings, because the present compounds do not exhibit the required estrogenic activity required by the '906 patent. Moreover, whatever estrogenic activity is viewed as being desirable by the '906 patent is completely inconsistent with its use in treating or reducing the likelihood of estrogen-sensitive cancer. Indeed, compounds having the required activity as taught by the '906 patent are essentially contraindicated (ie., worse than useless) in the presently claimed methods.

The '906 patent does not disclose or suggest that the compounds used in the present methods are active in treating osteoporosis, cholesterolemia or elevated LDL levels or cardiovascular disease or that the compounds could be used to treat or reduce the likelihood of breast cancer. Moreover, in treating these conditions/disease states, the compounds used in the present invention do not exhibit estrogenic activity in the uterus, vaginal area, brain or breast (anti-estrogenic activity) as required by the '906 patent, and the failure to exhibit such activity (in completely contravention to the teachings of the '906 patent), is actually beneficial to the treatment of the individual conditions or disease states, given that side effects associated with typical general estrogen therapy often result in unfavorable estrogen activity with increased endometrial cancer and breast cancer as side effects. In the present invention, unlike in the case of the prior art compounds, breast cancer is not a side effect, but its reduction is actually a favorable activity in the present invention. The present invention, therefore, represents a clear advance in the art because of the advantageous selective anti-estrogenic/estrogenic activity of the presently claimed compounds. It is respectfully submitted that evidence of non-obviousness of an invention is particularly strong where, as here, the use of the claimed invention completely contravenes the teaching of the cited art at the precise point of invention.

Consequently, it is respectfully submitted that the instantly claimed invention is clearly patentable over the '906 patent. There is no teaching or suggestion in the '906 patent (as confirmed by the attached JMedChem article using the assays of Jelinkova as previously

presented) of the presently claimed compounds or their selective activity in treating conditions secondary to menopause and estrogen-sensitive cancers. Finally there is absolutely no suggestion or motivation to use the compounds according to the present invention in the claimed methods, given that the compounds exhibit no estrogenic activity in assays typically used to assess such activity. The present invention is clearly patentable over the '906 patent. Applicant respectfully requests that the Examiner withdraw her rejection based upon the teachings of the '906 patent, inasmuch as that rejection is no longer cogent.

For the above reasons, Applicant respectfully asserts that the claims set forth in the amendment to the application of the present invention are now in compliance with 35 U.S.C. Applicants respectfully submit that the present application is now in condition for allowance and such action is earnestly solicited. Applicants have neither cancelled nor added any claim to the present application.

No claims have been added or deleted from the present application pursuant to the presentation of this amendment. Small entity status pertains to the present application. Enclosed is a petition for an extension of time (three months) and appropriate fee. If any additional fee is due or any overpayment has been made, please charge/credit Deposit Account No. 04-0838.

Should the Examiner wish to discuss this application in an effort to remove further issues and expedite prosecution of this application, she is cordially requested to telephone the undersigned attorney at the below-indicated telephone number.

November 20, 2008

Respectfully submitted,
Coleman Sudol Sapone, P.C.

By: _____
Henry D. Coleman
Regis. No. 32,559
714 Colorado Avenue
Bridgeport, Connecticut 06605-1601
(203) 366-3560

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# In Search of the Perfect SERM: Beyond Tamoxifen And Raloxifene

Never mind this spring's excitement over tamoxifen and raloxifene; they're just forerunners of designer estrogens to come. Second generation compounds are already in clinical trials. And with growing knowledge of how the estrogen receptor works, researchers say that new synthetic estrogens -- known as selective estrogen receptor modulators or SERMs -- will certainly be entering drug company pipelines over the next decade.

That long view may have been temporarily obscured by headlines emerging last month from the annual meeting of the American Society of Clinical Oncology in Los Angeles, where the two SERMs now on the market, tamoxifen (Nolvadex®) and raloxifene (Evista®), seemed to be competing stars.

Both drugs had slots at the plenary session and at an official ASCO press briefing. Reporting on tamoxifen was D. Lawrence Wickerham, M.D., associate director of the National Surgical Adjuvant Breast and Bowel Project, which conducted the Breast Cancer Prevention Trial. That trial showed that tamoxifen lowered the risk of breast cancer by 45% percent in high-risk women, compared to a placebo (see *News*, May 6, 1998).

A day before the plenary, tamoxifen's maker, Zeneca Pharmaceuticals of Wilmington, Del., had organized a hotel press conference that included high-profile spokespersons such as Harmon J. Eyre, M.D., of the American Cancer Society, and V. Craig Jordan, Ph.D., Northwestern University, Evanston, Ill., whose research led to the development of tamoxifen (see *News*, May 6, 1998). Both emphasized that the tamoxifen findings were based on mature, long-term data. In contrast, they said, much more research was needed on Indianapolis-based Eli Lilly's raloxifene before it could be recommended for the prevention of breast cancer.

For raloxifene, Steven Cummings, M.D., from the University of California at San Francisco, presented early data on the drug's ability to prevent breast cancer. After 2 years of followup among postmenopausal women taking it for osteoporosis, raloxifene appeared to lower the risk of breast cancer by 58% to 66% compared to a placebo. Moreover, it has so far not appeared to increase the risk of endometrial cancer, which is one of the drawbacks of tamoxifen.

The two SERMs are destined to meet head-to-head in a second breast cancer prevention trial, which will probably start recruiting patients this fall, according to Wickerham. The Study of

Tamoxifen and Raloxifene (STAR) will enroll about 22,000 post-menopausal women who will be randomly assigned to receive either one drug or the other, he added.

While tamoxifen-versus-raloxifene may have been the issue of the week, other ASCO speakers made it clear that eventually there should be more to the SERM story. "The perfect SERM . . . has not yet been developed," said C. Kent Osborne, M.D., of the University of Texas Health Science Center in San Antonio, the discussant for both the tamoxifen and raloxifene presentations. He added that "modern drug discovery techniques offer promise for its synthesis."

The perfect SERM would be a compound that acts as a potent anti-estrogen in the breast and uterus to prevent estrogen-driven cell proliferation and, at the same time, has strong estrogenic effects in bone, the cardiovascular system, and the central nervous system, where hormones can help prevent a variety of post-menopausal conditions.

**Second Generation**

It is not clear that anything approaching the perfect SERM is now in the pipeline, but a second generation of synthetic estrogens, most of them variations on tamoxifen and raloxifene, are in development.

At Eli Lilly, for instance, drug designers have taken raloxifene's structure as a starting point and are developing a variation that they call SERM III. Their aim, said Dapil Dhingra, M.D., an oncologist and clinical research physician at Lilly, is to optimize the drug's anti-estrogen effects in breast and endometrial tissue.

So far, in preclinical data, the compound does look like a more potent anti-estrogen than raloxifene, Dhingra said. In the clinic, two trials with SERM III are just getting under way; one is a phase II breast cancer treatment trial and one an early trial of the drug's ability to prevent breast cancer. Lilly is also planning a trial of SERM III to prevent osteoporosis.

**Perhaps a Preventive**

Another synthetic estrogen in the pipeline is droloxifene, being developed by Pfizer, Inc., New York City. The company originally envisioned droloxifene as a therapeutic drug in breast cancer, but interim data from a phase III trial was discouraging, showing that droloxifene "offered no benefit beyond the current therapy," according to Brian McGlynn, director of corporate media relations.

Pfizer had originally planned to file a New Drug Application for droloxifene with the Food and Drug Administration this year. Now it has dropped that plan, McGlynn said, and decided instead to evaluate droloxifene in the prevention of breast cancer and to accelerate development efforts in osteoporosis, where the data are more promising.

A third SERM in clinical trials is SmithKline Beecham's idoxifene, also designed as a variation on existing SERMs. It is now in a phase III trial for the prevention of osteoporosis and a phase II trial for the treatment of advanced breast cancer. The Philadelphia-based company says idoxifene

appears to be estrogenic in bone and anti-estrogenic in the breast, and so far does not increase the risk of endometrial hyperplasia.

If SERM III, droloxifene, idoxifene, and other tamoxifen-like drugs are considered second-generation SERMs, what will the the third generation be like?

"A fundamental change" is needed, said Donald P. McDonnell, Ph.D., a Duke University investigator who has been working on preclinical studies of a SERM called GW5638. (If GW5638 works out in clinical trials, it could offer an option for tamoxifen-resistant cancers, McDonnell said, but it too is basically a variation on current SERMs.)

The next generation of SERMs should be based on new knowledge about estrogen and estrogen receptor biology, McDonnell said, and that is turning out to be much more complex than once thought. For one thing, the different ligands, whether natural estrogens or SERMS, appear to interact with the receptor in different ways.

Knowing the intricacies of those interactions could help in designing new SERMs. For example, recent research using crystallography has clarified the way in which raloxifene and estradiol interact with the estrogen receptor. As Jordan points out in this issue (page 967), that knowledge has provided insight into the mechanisms of anti-estrogenic activity.

But SERM-receptor interactions are not the only events that need to be understood. There are a bevy of other molecules that get involved in a cell's response to estrogen. For instance, scientists recently discovered that there are actually two estrogen receptors, alpha and beta, that occur in different quantities in different cells and tissues. And, as Osborne pointed out, there are at least 20 different receptor interacting proteins that bind to the estrogen receptors and function either as co-activators to enhance estrogen's effect or as co-repressors to inhibit it.

"The estrogen receptor does not work in a vacuum," said McDonnell, speaking at a symposium last March in Chantilly, Va. "It has lots of dancing partners."

And that's not all. There are probably more than 50 transcription activating factors, TAFs, that interact to regulate the effects of estrogen on its target genes. Also at the DNA level, response elements in the promoter regions of the target genes may be involved in the complex process that determines what effect a given SERM will have on tissues.

"Many biologists now feel it is the particular ensemble of ligands, receptors, receptor interacting proteins, and response elements, that determine whether there will be a predominantly agonist or antagonist signal on a given tissue or gene," said Osborne. One of the challenges facing the designers of third-generation SERMs will be defining the workings of these ensembles and developing drugs that target them.

**Multiple Options**

The third-generation drugs that emerge from this process may be some years away. But the intense interest in SERMs makes it seem certain that eventually clinicians can expect to have an increasing number of options.

"It will be like antibiotics," said Jordan, envisioning a time when there will be many more than two SERMs on the market. In an interview at ASCO, he compared tamoxifen and raloxifene to penicillin, which over the years has been joined by a host of other antibiotics, each with its own indications. One SERM is not necessarily going to replace another, he predicted. Instead, "there will be a menu of options for specific subsets of patients."

-- *Caroline McNeil*

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# Randomized clinical trials of raloxifene: reducing the risk of osteoporosis and breast cancer in postmenopausal women

*Olga M. Olevsky, MD, and Silvana Martino, DO*

**Abstract**

The P-1 trial of tamoxifen versus placebo for breast cancer prevention in high-risk women provided proof of the concept that chemoprevention was effective. The approval of tamoxifen for this indication in 1998 provided a pharmaceutical agent as an alternative to bilateral mastectomy and/or oophorectomy for women at high risk. Although for some women and their physicians this was a welcome alternative, fear of toxicities from tamoxifen has been a major barrier to its use. Four trials evaluating the role of raloxifene in breast cancer prevention have resulted in the recent approval by the US Food and Drug Administration for postmenopausal women with osteoporosis or at high risk of breast cancer. It has a more favorable toxicity profile than tamoxifen and provides an alternative for postmenopausal women.

*Key Words:* Raloxifene – Osteoporosis – Breast cancer – Postmenopausal women.

Breast cancer remains the most frequently diagnosed cancer in American women. In 2007 an estimated 178,840 new cases of invasive breast cancer and 62,030 additional cases of in situ breast cancer will be diagnosed. The expected number of new breast cancer cases in 2007 is lower owing to a new, more accurate estimation method and a small decline in the breast cancer incidence rate.[1] In 2007, approximately 40,460 women are expected to die from breast cancer. Breast cancer is the second leading cause of cancer deaths in American women and accounts for 15% of all cancer deaths among women. Only lung cancer accounts for more cancer deaths in women.[2]

It is increasingly apparent that breast cancers are not all the same. Breast cancer may be a group of diseases with only some overlapping features and with many distinctive features. The distinctions may have their genesis in different cellular elements within the breast that give rise to clinically different breast cancer subtypes.[3] This new understanding is important for the development of targeted therapies for invasive breast cancer. It is likely that it will also contribute to the development of strategies for breast cancer prevention. One clear distinction is that 70% to 80% of breast cancers are hormone receptor–positive, whereas the remainder are not. The hormone-dependent nature of most breast cancers has made hormone therapy a logical choice for investigating prevention strategies.

The role of the estrogen receptor mechanism in the development of breast cancer is well recognized. Cells that are a target for estrogen contain one or both species of estrogen receptors α and β. Estrogen receptor α is almost always an activator, whereas estrogen receptor β is an antagonist. The relative level of these receptors in a cell affects the cellular response to estrogen.

Unlike estrogens that are uniformly agonists or antiestrogens that are uniformly antagonists, selective estrogen receptor modulators (SERMs) exert differential effects on various estrogen target tissues. Their structure allows them to bind to the estrogen receptor and change its conformation in different ways. This process enables the complex to bind corepressor proteins and thus inhibit estrogen activity in some tissues, such as the breast. Binding to coactivator proteins and subsequent interactions with estrogen response elements in target genes leads to agonist-like effects in bone and lipid metabolism.[4]

Until September 2007, tamoxifen, a triphenylethylene SERM was the only drug approved by the Food and Drug Administration for the prevention of breast cancer in women at high risk for the disease. Its effects were demonstrated in the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 trial conducted by Fisher et al[5] and confirmed by other studies.[6-8] However, multiple adverse events were observed when tamoxifen was administered to this group of women. There was increased incidence of venous thrombotic events (VTEs), endometrial cancer, and strokes. Raloxifene, a benzothiophene SERM that is chemically distinct from tamoxifen, seems to cause fewer adverse events. It has been studied for the prevention of both osteoporosis and breast cancer.

In this article we summarize three randomized, placebo-controlled trials (Multiple Outcomes of Raloxifene Evaluation

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From The Angeles Clinic and Research Institute, Santa Monica, CA.

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Address correspondence to: Silvana Martino, DO, The Angeles Clinic and Research Institute, 2001 Santa Monica Boulevard, Suite 560W, Santa Monica, California 90404. E-mail: smartino@theangelesclinic.org

[MORE], Continuing Outcomes Relevant to Evista [CORE], and Raloxifene Use for the Heart [RUTH]) and Study of Tamoxifen and Raloxifene (STAR), a randomized trial comparing tamoxifen and raloxifene, in which the effects of raloxifene on breast cancer prevention have been evaluated.

## MORE

The MORE trial had a 3-year treatment phase plus a 1-year extension study and was conducted from 1994 through 1998. It was a double-blind, placebo-controlled trial performed in 180 clinical centers in 25 countries.

Participants were at least 2 years postmenopausal and osteoporotic. Osteoporosis was defined as the presence of radiographically apparent vertebral fractures or bone mineral density T score less than −2.5. Exclusion criteria included the presence of known or suspected breast cancer, invasive endometrial cancer, abnormal uterine bleeding, history of stroke or venous thromboembolism, or the presence of other cancers. Women were also excluded if they used systemic or topical estrogens, progestins, androgens, or systemic corticosteroids or if they used ≥50,000 IU of cholecalciferol per week or drank ≥4 alcohol beverages per day. The primary endpoint of the trial was the determination of the effect of raloxifene therapy on the risk of vertebral and nonvertebral fractures in postmenopausal women with osteoporosis.

A total of 7,705 women aged 31 to 80 years (mean age 66.5 y) were randomly assigned. One-third (n = 2,576) were randomly assigned to placebo and two-thirds (n = 5,129) were randomly assigned to raloxifene. Of those randomly assigned to raloxifene, half (n = 2,557) received 60 mg/d and half (n = 2,572) received 120 mg/d. Women were followed every 6 months. Mammograms and transvaginal ultrasonography were performed annually. All possible VTEs were reported, and other possible adverse events were reviewed at each visit. Participants in all study groups were evenly matched with respect to age, sex, race, and mean body mass.

At 36 months of observation, the risk of vertebral fractures was reduced in both groups receiving raloxifene (for the 60 mg/d group: relative risk [RR] = 0.7; 95% CI: 0.5-0.8; for the 120 mg/d group: RR = 0.5; 95% CI: 0.4-0.7). Vertebral fractures were reduced in both women who did and did not have a prevalent fracture at study entry. However, the risk of nonvertebral fractures for raloxifene versus placebo did not differ significantly (RR = 0.9; 95% CI: 0.8-1.1) for both raloxifene groups combined. Compared with placebo, raloxifene increased bone mineral density in the femoral neck by 2.1% in the 60 mg/d group and by 2.4% in the 120 mg/d group and in the spine by 2.6% in the 60 mg/d group and by 2.7% in the 120 mg/d group. These differences were statistically significant compared to placebo ($P < 0.001$). There was no statistically significant difference between the 2 doses of raloxifene, and therefore the 120 mg/d dose did not seem to offer an advantage.[9] With an additional year of observation, the overall results remained the same.[10] These data resulted in the approval of raloxifene at 60 mg/d for the treatment and prevention of osteoporosis.

Breast cancer incidence was one of several secondary endpoints of the MORE trial. To evaluate this endpoint, mammograms or breast ultrasound examinations were obtained at baseline, and mammograms were subsequently obtained at years 2, 3, and 4. All investigator-reported breast cancers were reviewed and adjudicated by a board of breast cancer experts who were blinded to treatment assignment. The result of the MORE trial demonstrated that raloxifene reduced the risk of invasive breast cancer by 76% after 3 years (RR = 0.24; 95% CI: 0.13-0.44) and by 72% after 4 years (RR = 0.28; 95% CI: 0.17-0.46). It further demonstrated that this benefit was specific to estrogen receptor–positive (ER+) breast cancer, for which an 84% reduction was noted (RR = 0.16; 95% CI: 0.09-0.30). There was no effect on risk of estrogen receptor–negative breast cancer. In addition, there were no significant differences noted between the two doses of raloxifene (Table 1).

An increased risk of VTEs, including deep vein thrombosis and pulmonary embolism, relative to placebo was apparent (RR = 3.1; 95% CI: 1.5-6.2). There was no difference in the incidence of endometrial cancer. The more common side effects seen in women treated with raloxifene were influenza-like symptoms, hot flashes, leg cramps, and peripheral edema.[11,12]

## CORE

The favorable breast cancer results evident in the MORE trial motivated a decision to enroll as many women as possible into a subsequent follow-up study known as the CORE trial. The primary objective of CORE was to determine the incidence of invasive breast cancer in response to raloxifene for an additional 4-year period beginning January 1, 1999. Secondary objectives were to determine the effect of raloxifene on the incidence of invasive ER+ breast cancer and evaluate toxicities associated with raloxifene over an 8-year period.

At the conclusion of the MORE trial, participants were screened and consented to participation in CORE. Women enrolled in CORE had at least a brief period of no treatment with raloxifene with the median time between the end of participation in MORE and enrollment in CORE of 10.6 months for all treatment groups.

**TABLE 1.** *Incidence of invasive breast cancer in MORE, CORE, and RUTH trials*

| Trial | Placebo | Raloxifene | Hazard ratio (95% CI) | *P* value |
|---|---|---|---|---|
| MORE | | | | |
| No. of subjects | 2,576 | 5,129 | 0.28 (0.17-0.46) | <0.001 |
| No. of invasive breast cancer | 39 | 22 | | |
| CORE | | | | |
| No. of subjects | 1,703 | 3,510 | 0.41 (0.24-0.71) | <0.001 |
| No. of invasive breast cancer | 28 | 24 | | |
| RUTH | | | | |
| No. of subjects | 5,057 | 5,044 | 0.56 (0.38-0.83) | 0.003 |
| No. of invasive breast cancer | 70 | 40 | | |

Adapted from Cauley et al,[12] Martino S et al,[13] and Barrett-Connor et al.[15]
MORE, Multiple Outcomes of Raloxifene Evaluation; CORE, Continuing Outcomes Relevant to Evista; RUTH, Raloxifene Use for the Heart.

The original MORE trial had three treatment groups: placebo, raloxifene at 60 mg/d, and raloxifene at 120 mg/d. Women who enrolled in CORE continued the treatment regimen to which they had previously been randomly assigned. The dose of raloxifene, however, was the approved dose of 60 mg/d and twice as many women were assigned to raloxifene as to placebo.

Only 130 of the 180 MORE investigators agreed to participate in CORE, contributing 4,011 women who were recruited for the CORE trial. An additional group of 1,217 women at these sites were completing their MORE participation when CORE was being designed. By study design, their breast cancer data were encompassed and reported as part of the CORE time period, which began on January 1, 1999. This date represented the starting point at which invasive breast cancer reduction became the primary endpoint. In 15 women, breast cancer had been identified prior to the January 1, 1999 date, and were excluded. Therefore, the CORE breast cancer analysis was based on 5,213 participants.

The CORE trial continued as a double-blind, placebo-controlled trial. Clinical breast examinations and collection of toxicity data occurred at yearly visits. Mammography was performed at baseline and at years 2 and 4. Breast cancer data were adjudicated by an independent physician review board composed of a medical oncologist, a pathologist, and a radiologist, who were blinded to treatment assignment. Breast cancer results were analyzed by intent-to-treat analysis.

Fifty-two cases of invasive breast cancer (28 in the placebo group and 24 in the raloxifene group) were reported and confirmed by adjudication. During the 4 years of the CORE trial, the incidence of invasive breast cancer was reduced in the raloxifene-treated group by 59% compared with that in the group of women randomly assigned to placebo (hazard ratio [HR] = 0.41; 95% CI: 0.24-0.71). Again, the reduction in breast cancer seemed to be specific to ER+ breast cancer with a 66% reduction (HR = 0.34; 95% CI: 0.18-0.66) and no significant differences between the placebo and raloxifene groups with respect to estrogen receptor–negative breast cancer. In addition, no significant difference was noted for the incidence of noninvasive breast cancer.

When data from the 8-year period including both the MORE and CORE trials are combined, raloxifene reduced the incidence of invasive breast cancer by 66% (HR = 0.34; 95% CI: 0.22-0.50) and that of invasive ER+ breast cancer by 76% (HR = 0.24; 95% CI: 0.15-0.40). Therefore, the results of the MORE trial, the CORE trial, and the combined 8-year analysis were consistent and demonstrated a reduction in the risk of invasive breast cancer in those treated with raloxifene compared with placebo.[13]

There were no additional toxicities associated with raloxifene during the CORE trial. There were no statistically significant differences between the placebo and raloxifene groups with respect to rates of vaginal bleeding, endometrial hyperplasia, or endometrial cancer for the 8-year study analysis. A total of 89 benign uterine polyps were reported, with an incidence of 3.2% versus 1.9% in the raloxifene and placebo groups, respectively. Nearly twice as many VTEs were reported for women in the raloxifene group. The highest event rate was noted during the first 4 months of therapy, and the rate remained significantly higher for the entire 8-year period. The difference in rates of pulmonary emboli was significant ($P = 0.048$) between the placebo group (0.2%) and the raloxifene group (0.6%). Flushing was more common in the raloxifene group with an incidence of 12.6% versus 6.9% in the placebo group ($P < 0.001$). Similarly, muscle cramps were reported more often by those receiving raloxifene (14.9% vs 11.8%; $P = 0.008$). Death of any cause, ovarian cancer, strokes, myocardial infarctions, and breast symptoms were not significantly different between the two groups[14] (Table 2).

**RUTH trial**

Prior studies suggested that raloxifene therapy was associated with improved levels of serum lipoprotein cholesterol, fibrinogen, and homocysteine. The RUTH trial was designed to evaluate the benefits and risks of raloxifene treatment in women with or at increased risk for coronary heart disease (CHD). RUTH was an international, multicenter, randomized, double-blind, placebo-controlled trial with two primary endpoints. The first endpoint evaluated the effect of raloxifene versus placebo on the incidence of coronary events (death of coronary causes or infarction or hospitalization for acute coronary syndromes). The second primary endpoint was the effect of raloxifene on rates of invasive breast cancer.

From June 1988 to August 2000, 10,101 postmenopausal women were randomly assigned to receive placebo or raloxifene at 60 mg/d. Eligible women were 55 years or older, were postmenopausal, and had CHD or were at risk for development of CHD. Risk of CHD was graded on a four-point system and a score of at least four points was required to be eligible for the study. The four-point system consisted of the following: established CHD (four points), arterial disease of the leg (four points), age 70 years or older (two points), diabetes mellitus (three points), current cigarette smoker (one point), hypertension (one point), and hyperlipidemia (one point). At intervals of 6 months, visits or telephone interviews were conducted with participants who were questioned about adverse events and adherence to study medication. Electrocardiograms were performed at baseline, at years 2 and 4, and at the final visit. Mammography and breast examination were performed at randomization and every 2 years thereafter. Serum lipids were evaluated at baseline, at years 1 and 5, and at the final visit. Seventy-one percent of the women in the placebo group and 70% of the women in the raloxifene group took 70% of their assigned medication.

With a median follow-up duration of 5.6 years, there was no significant difference between the raloxifene and placebo groups in the incidence of death from coronary causes, nonfatal myocardial infarction, or hospitalization for acute coronary syndromes (HR = 0.95; 95% CI: 0.84-1.07) (Fig. 1). The effect of raloxifene was not different in women with established

TABLE 2. *Summary of adverse outcomes for the 4,011 participants of MORE and CORE over 8 years*

| | Percentage (n) of participants who experiences event | | |
|---|---|---|---|
| | Placebo (N = 1,286) | Raloxifene (N = 2.725) | $P^a$ |
| Mortlity | 2.3 (29) | 1.7 (47) | 0.27 |
| All cancers[b] | 8.6 (110) | 5.7 (156) | 0.001 |
| All cancers excluding breast cancer[b] | 6.3 (81) | 4.6 (126) | 0.027 |
| Hospitalization | 40.9 (526) | 38.8 (1057) | 0.21 |
| Treatment-emergent AEs | 99.0 (1273) | 98.6 (2688) | 0.45 |
| Treatment-emergent serious AEs | 45.5 (585) | 42.3 (1154) | 0.07 |
| All venous thromboembolic events | 1.01 (13) | 1.72 (47) | 0.094 |
| Pulmonary embolism | 0.16 (2) | 0.62 (17) | 0.048 |
| Deep vein thrombosis | 0.78 (10) | 1.14 (31) | 0.32 |
| Retinal vein thrombosis | 0.16 (2) | 0.22 (6) | >0.99 |
| Uterine cancer[c,d] | 0.39 (4) | 0.32 (7) | 0.75 |
| Endometrial hyperplasia[d] | 0.29 (3) | 0.37 (8) | >0.99 |
| Ovarian cancer | 0.16 (2) | 0.11 (3) | 0.66 |
| Postmenopausal bleeding[d,e] | 5.4 (55) | 5.5 (120) | 0.87 |
| Uterine polyps[d] | 1.9 (19) | 3.2 (70) | 0.028 |
| Vulvovaginal signs and symptoms | 5.8 (75) | 5.0 (135) | 0.26 |
| Muscle cramps | 11.8 (152) | 14.9 (407) | 0.008 |
| Flushing | 6.9 (89) | 12.6 (342) | <0.001 |
| Biopsy endometrium[d] | 3.9 (40) | 6.8 (148) | <0.001 |

*Fisher's exact test.
[a]Fisher's exact test.
[b]Excluding nonmelanoma skin cancers.
[c]Eleven uterine cancers comprising 10 endometrial cancers and 1 uterine sarcoma.
[d]Based on 3,193 participants with an intact uterus at MORE baseline (placebo, 1,026; raloxifene, 2,167).
[e]Comprises events classified under the preferred terms "postmenopausal hemorrhage," "vaginal hemorrhage," "uterine hemorrhage," "metrorrhagia," "genital hemorrhage," and "dysfunctional uterine bleeding."
Adapted from: Martino S, Disch D, Dowsett SA, Keech CA, Mershon JL. Safety assessment of raloxifene over eight years in a clinical trial setting. Current Medical Research and Opinion 2005;21(9):1441-1452 (Reference number 14).

coronary disease compared with women without coronary disease. Raloxifene did, however, lower the risk of invasive breast cancer by 44% (HR = 0.56; 95% CI: 0.38-0.83; $P$ = 0.003). Hormone receptor–positive breast cancer was reduced by 55% in women treated with raloxifene (HR = 0.45; 95% CI: 0.28-0.72; $P \leq 0.001$) but there was no reduction in the risk of hormone receptor–negative invasive breast cancer.

There were 64 clinical vertebral fractures in those treated with raloxifene and 97 in the placebo group resulting in a 35% risk reduction (HR = 0.65; 95% CI: 0.47-0.89; $P$ = 0.007). There was no significant reduction in nonvertebral fractures,

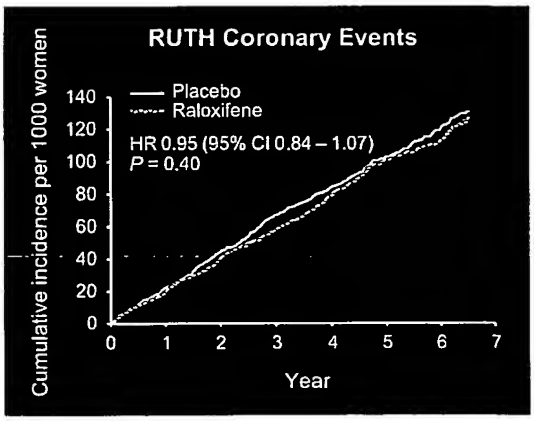


FIG. 1. Cumulative Incidence of the Primary Outcome of Coronary Events (Death from Coronary Causes, Nonfatal Myocardial Infarction, or Hospitalization for an Acute Coronary Syndrome Other Than Myocardial Infarction). Adopted from Barret-Connor et al.[15]

with 428 events reported in the raloxifene group versus 438 in the placebo group (HR = 0.96; 95% CI: 0.84-1.10; $P$ = 0.59).

Analysis of adverse events demonstrated that the overall incidence of stroke did not differ significantly between the two groups with 249 events in the raloxifene group and 224 in the placebo group ($P$ = 0.30). However, the incidence of fatal stroke was 49% higher in the raloxifene group, with 59 deaths attributable to cerebrovascular events compared with 39 in the placebo group (HR = 1.49; 95% CI: 1.00-2.24; $P$ = 0.05). The incidence of VTEs was 44% higher in the raloxifene group than in the placebo group (HR = 1.44; 95% CI: 1.06-1.95; $P$ = 0.02). The incidence of non–breast cancers, including endometrial cancer, did not differ significantly between the two groups ($P$ = 0.79) (Table 3). Hot flashes, arthritis, cholelithiasis, dyspepsia, muscle spasm, and peripheral edema were more common in the raloxifene group than in the placebo group ($P \leq 0.05$).[15]

## STAR

The STAR trial was conducted by the NSABP to directly compare tamoxifen to raloxifene in postmenopausal women at increased risk of breast cancer. Details of this trial are discussed by Rastogi in this issue.[16]

Results demonstrated no difference between the tamoxifen group and raloxifene group in the incidence of invasive breast cancer with 163 versus 168 events, respectively (RR = 1.02; 95% CI: 0.82-1.28) (Fig. 2). There were fewer noninvasive cancers (both lobular carcinoma in situ and ductal carcinoma in situ) in the tamoxifen group than in the raloxifene group, with a rate of 1.5 per 1,000 in the

TABLE 3. *Summary of endometrial cancer in women with an intact uterus in MORE, CORE, 8-year MORE and CORE, and RUTH trials*

| Trial | No. with uterus | Placebo, n (%) | Raloxifene 60 mg, n (%) | Raloxifene 120 mg, n (%) | *P* |
|---|---|---|---|---|---|
| MORE | 5,959 | 5 (0.30) | 5 (0.30) | 4 (0.2) | 0.86[a] |
| CORE | 3,146 | 3 (0.30) | 4 (0.19) | — | 0.69 |
| 8-y MORE and CORE | 3,193 | 4 (0.39) | 7 (0.32) | — | 0.75 |
| RUTH | 7,782 | 17 (0.4) | 21 (0.50) | — | 0.53 |

Adapted from Cauley JA, Norton L, Lippman ME, Eckert S, Krueger KA, Purdie DW, Farrerons J, Karasik A, Mellstrom D, Ng KW, Stepan JJ, Powles TJ, Morrow M, Costa A, Silfen SL, Walls EL, Schmitt H, Muchmore DB, Jordan VC. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Breast Cancer Research and Treatment 2001;65:125-134.
Martino S, Cauley JA, Barrett-Connor E, Powles TJ, Mershon J, Disch D, Secrest RJ, Cummings SR. Continuing Outcomes Relevant to Evista: Breast Cancer Incidence in Postmenopausal Osteoporotic Women in a Randomized Trial of Raloxifene. J Natl Cancer Inst 2004;96(23:1751-1761.)
Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Komitzer M, McNabb MA, Wenger NK. Effects of Raloxifene on Cardiovascular Events and Breast Cancer in Postmenopausal Women. N Engl J Med 2006;355:125-137.

tamoxifen group and 2.1 per 1,000 in the raloxifene group (RR = 1.40; 95% CI: 0.98-2.00). Of these, 36% were lobular carcinoma in situ and 54% were ductal carcinoma in situ, with the remaining 10% being mixed types. This difference did not reach statistical significance ($P = 0.05$). However, it is in accord with similar observations made in the MORE and CORE trials. Within each of these trials, the incidence of noninvasive breast cancer was small, therefore limiting the ability to demonstrate statistical significance. The clinical impact of this finding remains unclear, as does a plausible biological explanation.

The incidence of uterine cancer was lower in the raloxifene group compared with that in the tamoxifen group, although this difference did not reach statistical significance. Annual incidence was 2 per 1,000 in the tamoxifen group and 1.2 per 1,000 in the raloxifene group (RR = 0.62; 95% CI: 0.35-1.08). Only one case of uterine cancer was reported in a woman younger than age 50 who was in the tamoxifen group. The rate of uterine hyperplasia was 84% lower with 14 cases in the raloxifene group versus 84 cases with tamoxifen (RR = 0.16; 95% CI: 0.09-0.29). Among women who did not have a diagnosis of endometrial cancer, 244 hysterectomies were performed in the tamoxifen group compared with 111 for women treated with raloxifene (RR = 0.44; 95% CI: 0.35-0.56). There was no statistically significant difference between groups in the incidence of any other cancers with colorectal, lung, and hematological cancers being the most frequently diagnosed non–breast cancer malignancies. There was also no difference between the two treatment arms in the incidence of ischemic heart disease (myocardial infarction, severe angina, and acute ischemic syndromes) and no significant differences were reported for strokes (53 with tamoxifen and 51 with raloxifene) and transient ischemic attacks between the two groups. Only 10 deaths were attributed to stroke, with six of these occurring in the tamoxifen group and four in women treated with raloxifene. There was a statistically significant difference in the incidence of VTEs, with 141 events in the tamoxifen group and 100 events in the raloxifene group (RR = 0.70; 95% CI: 0.54-0.91). Fewer pulmonary emboli (35) were reported in the raloxifene group than in the tamoxifen group (54) (RR = 0.64; 95% CI: 0.41-1.00). Similarly, fewer deep vein thromboses (65 vs 87) occurred in the raloxifene group than in the tamoxifen group (RR = 0.74; 95% CI: 0.53-1.03).

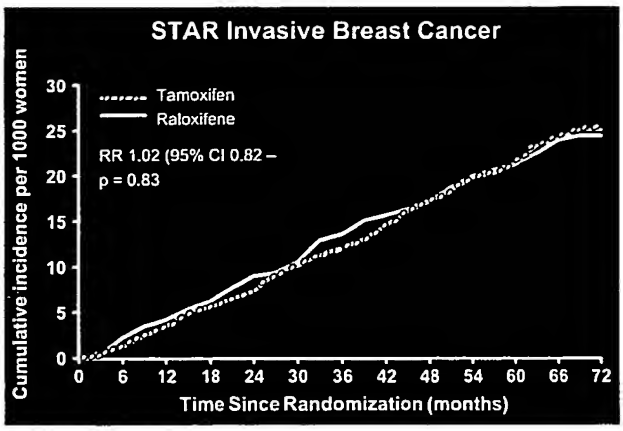


FIG. 2. Cumulative Incidence of Invasive Breast Cancer. Adopted from Vogel et al.[17] *JAMA* 2006;295:2727-2741, Copyright 2006, American Medical Association. All rights reserved.

There were no statistically significant differences between the two study groups in the numbers of fractures of the hip, spine, and wrist (Colles fractures), which were sites prespecified for monitoring. Fewer cataracts (RR = 0.79; 95% CI: 0.68-0.92) and cataract operations (RR = 0.82; 95% CI: 0.68-0.99) were reported for the raloxifene group than for the tamoxifen group. Finally, mortality rates were similar between the two treatment groups (RR = 0.94; 95% CI: 0.71-1.26).

In summary, the STAR trial demonstrated that raloxifene and tamoxifen have equivalent efficacy for reduction of risk for invasive breast cancer in postmenopausal women who are at an increased risk. Unlike tamoxifen, raloxifene may not decrease the risk of noninvasive breast cancer. In addition, raloxifene was associated with fewer endometrial cancers, VTEs, and cataracts than tamoxifen.

No statistically significant difference in overall quality of life was found between the two groups. Women in the tamoxifen arm experienced more vasomotor symptoms, vaginal discharge, vaginal bleeding, difficulty with urinary bladder control, and leg cramps. Self-reported sexual function was better among women treated with tamoxifen. Pain with intercourse was more common among women who received raloxifene, as was self-reported weight gain and musculoskeletal discomfort.[17]

## DISCUSSION

The concept that breast cancer might be preventable by a systemic treatment first arose with the observation of decreased

breast cancer in premenopausal women who had an oophorectomy.[18,19] The second observation was made in women who received adjuvant therapy with tamoxifen in whom a lower rate of contralateral breast cancers was observed.[20] These two observations led to the concept that breast cancer might be preventable by a systemic treatment. The acceptability of an orally administered agent rather than a surgical or injectable therapy (ie, leuprolide or goserelin) made tamoxifen a reasonable first agent with which to test this hypothesis, thus leading to the P-1 trial conducted by the NSABP.

Underlying this effort was a plausible biological assumption that all breast cancers are hormone dependent at the beginning and that as they develop and progress some acquire hormonal independence. Based on this premise, hormone therapy would decrease all breast cancers, assuming that its protective effects occurred early in the development of the cancer process. The alternative hypothesis was that breast cancers were hormone dependent or hormone independent from the beginning. In this case, only hormone-dependent cancers would be affected by hormone therapies and hormone-independent cancers would not.

The results from the P-1 trial suggested that the distinction between hormone-dependent and -independent cancers occurs early rather than late in the process of cancer development. Thus, only hormone-positive breast cancers would decrease in number, and there would be no decrease in the number of estrogen receptor–negative cancers. The P-1 trial confirmed the hypothesis that systemic hormone therapy could reduce the incidence of hormone-positive breast cancer.

The next logical inquiry then became, was there a better therapeutic option than tamoxifen, which was associated with significant toxicities? Raloxifene became a candidate because of its basic biological mechanism as a SERM with the potential to reduce the risk of some of the adverse events caused by tamoxifen.

Three placebo-controlled trials (MORE, CORE, and RUTH) were conducted to evaluate the efficacy and safety of raloxifene. The first two trials evaluated a patient population at high risk for osteoporosis. In fact, the CORE study sample was a subgroup of participants in the MORE trial and not truly a separate group. The RUTH trial enrolled a separate population selected on the basis of their increased risk of CHD. In each of these three trials, raloxifene significantly reduced rates of invasive breast cancer relative to placebo. Thus, the hypothesis that breast cancer can be prevented by a systemic hormone therapy was confirmed. These studies also reaffirmed the fact that risk reduction is confined to hormone receptor–positive breast cancers, presumably those of luminal origin.

The final question is which agent is superior for prevention of breast cancer: raloxifene or tamoxifen? This is the question posed in the STAR trial. As in P-1, the STAR trial studied a population of women at high risk for breast cancer. Unlike P-1, all STAR participants were postmenopausal. The results of the STAR trial suggest several important conclusions. First, the two agents are similar in reducing invasive breast cancer risk. Second, hormone receptor–negative breast cancer is not affected by either agent. Third, less overall toxicity occurs with raloxifene. In addition, a fourth conclusion may be warranted. Although the numbers are small and the data are inconclusive, it does seem that tamoxifen may be more effective for reducing the risk of noninvasive breast cancer. This finding remains perplexing and implies some inferiority of raloxifene. This result should not be confused with the finding that both agents are equally as effective at decreasing the development of invasive breast cancer in women presenting with lobular carcinoma in situ and ductal carcinoma in situ. Another clear advantage of tamoxifen is its effectiveness in both pre- and postmenopausal women as demonstrated in the P-1 trial. At present, the data with raloxifene are limited to women who are postmenopausal.

Raloxifene offers the advantage of a more favorable toxicity profile. The three placebo-controlled trials demonstrated no significant increase in endometrial cancer, cataracts, or strokes. An increase in stroke deaths was reported only in the RUTH trial. The explanation for this finding is not clear. It may reflect comorbidity in this population, which was selected on the basis of increased risk of CHD. Alternatively, it may reflect a more specific and detailed attempt to capture this parameter in the RUTH trial versus the MORE and CORE trials. Compared with tamoxifen, raloxifene is associated with significantly fewer vascular events, less discontinuation of therapy from side effects in general, and a lower number of hysterectomies. The number of hysterectomies performed for benign causes in the STAR trial increased twofold among women treated with tamoxifen. Endometrial hyperplasia, a risk factor for endometrial cancer, was more common in the tamoxifen group, suggesting that the activity of these two agents on the uterus are different. The rates of uterine cancer were 2 per 1,000 in the tamoxifen group versus 1.25 per 1,000 for women treated with raloxifene. Although this difference was not statistically significant, it is likely that the rate of benign hysterectomies resulted in an underestimate of this event.

Hot flashes remain an issue with both drugs. The unpleasantness of this side effect is generally underappreciated. An agent without this side effect would be a clear advantage to women.

Criticisms of the STAR trial have centered around two issues: the lack of a placebo control group and the fact that STAR was not designed as either a true superiority or a noninferiority trial. The answers to these criticisms are rooted in practicality. Because tamoxifen was available and approved for breast cancer prevention, many felt offering a placebo to high-risk women would have been unethical. Accrual would have suffered as high-risk women would have been reluctant to accept random assignment placebo. A noninferiority design or a superiority design based on breast cancer incidence was calculated to require 80,000 participants and would have been impractical.

Underlying the entire concept of breast cancer prevention, irrespective of which agent (tamoxifen or raloxifene) one

considers best, is a general concern of whether either agent is good enough or safe enough to be recommended at all. Tamoxifen was approved for anyone with a 5-year predicted Gail score ≥1.67%. This includes any woman aged 60 or older. However, limited use has been made of this agent in primary prevention. Presumably, this has been attributable to its toxicity and the fact that tamoxifen is perceived as a cancer drug by most physicians, with its use relegated to oncologists. Because raloxifene has fewer serious side effects and is used by primary care physicians, internists, and gynecologists as a treatment for osteoporosis, it is possible, but not certain, that it will be prescribed more broadly. Like tamoxifen, raloxifene is indicated for prevention of invasive breast cancer in postmenopausal women at high risk for breast cancer. Unlike tamoxifen, raloxifene has a second indication for reduction of breast cancer risk in postmenopausal women with osteoporosis irrespective of breast cancer risk. Raloxifene, however, does not have an indication for risk reduction of noninvasive breast cancer.

An important criticism of all trials performed to date is that none have been designed to answer the question of whether survival is influenced by either agent. It has been argued that reducing breast cancer incidence alone is a sufficient benefit given the serious nature of the disease. However, as there are no data defining the length of therapy or at what age this therapy should be used, clinicians must recognize the potential for long-term consequences that may decrease survival.

The role of hormone therapy in reducing the development of breast cancer continues to evolve. A newer class of hormones, the aromatase inhibitors, is now being studied, and in time they may prove to be more effective than SERMs.[21] If these agents are effective, their use is likely to also be restricted to postmenopausal women for reduction of hormone receptor–positive disease. Continued thought must be directed to identifying ways to reduce breast cancer risk in premenopausal women and in those with hormone receptor–negative breast cancer.

## CONCLUSIONS

The management of breast cancer and breast cancer risk remains dynamic. With improved detection and treatment, breast cancer death rates have declined in the United States. The prevention of breast cancer remains the ultimate goal. For women at particularly high risk, such as those who are carriers of the *BRCA1* or *BRCA2* mutation, bilateral mastectomy and/or bilateral salpingo-oophorectomy may be reasonable options. For all other women, more acceptable prevention strategies are preferred. Both tamoxifen and raloxifene offer risk reduction in selected populations. Women using these agents must be closely monitored for potential side effects associated with these agents.

Where do we go from here? The proposed P-4 Breast Cancer Prevention Study comparing raloxifene with letrozole has been placed on hold by the National Cancer Institute. We acknowledge that the cost of large randomized cancer prevention clinical trials is high, yet it seems unlikely that we will move forward without them.

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# Alternate Antiestrogens and Approaches to the Prevention of Breast Cancer

V. Craig Jordan, PhD, DSc

Robert H. Lurie Cancer Center, Northwestern University Medical School, Chicago, IL 60611

**Abstract** The biological rationale and extensive clinical experience with the breast cancer drug tamoxifen make it the agent of choice for *testing* as a breast cancer preventive. However, concerns (Jordan and Morrow, *Eur J Cancer*, in press) about development of endometrial cancer in patients and liver tumors in rats with tamoxifen has encouraged the investigation of other antiestrogens. At present no compounds are available to replace tamoxifen, but two triphenylethylenes, toremifene and droloxifene, have been tested in postmenopausal women to treat advanced breast cancer. The response rates are similar to those observed with tamoxifen (*i.e.*, approximately 35% [CR + PR] in unselected patients), although dosage regimens of the new antiestrogens are higher than the 20 mg tamoxifen required daily. Doses of up to 200 mg toremifene daily are being tested and studies use up to 100 mg droloxifene daily. Side effects appear comparable, but neither droloxifene nor toremifene produce liver tumors in rats. Tamoxifen produces DNA adducts, whereas toremifene and droloxifene appear to be only weakly active. A new tamoxifen analogue, idoxifene, is entering clinical trial. The drug is designed to be metabolically stable so that there will be low carcinogenic potential.

In contrast, a novel strategy may be considered to be of value to protect women from developing breast cancer. It is known from laboratory and clinical studies that antiestrogens protect bone and prevent rat mammary cancer. One compound, raloxifene, is being tested as an agent to treat osteoporosis. If the drug becomes generally available to prevent osteoporosis in postmenopausal women, a beneficial side effect may be a reduction in breast cancer risk. This broad-based strategy may prove more effective than focusing on small groups of women with a high risk for breast cancer alone. Protection from breast cancer may be as an advantageous side effect from the successful treatment of other diseases in women. 

Key words: Breast cancer, droloxifene, endometrial cancer, liver cancer, raloxifene, tamoxifen, toremifene

Tamoxifen (Fig. 1) is the endocrine therapy of choice for selected patients with all stages of breast cancer [1]. An overview analysis [2] demonstrates a survival advantage for both node-positive and node-negative patients who received adjuvant tamoxifen therapy. Tamoxifen can reduce the incidence of fatal myocardial infarction [3,4] and stabilize bone density in postmenopausal patients [5–7], an incentive to test its worth as a preventive in women who are only at risk for breast cancer [8,9].

Tamoxifen has a low incidence of side effects [1], but its balance of estrogenic and antiestrogenic action that is considered an advantage [9] may result in more serious complications. Concerns about endometrial carcinoma [10–13] and developing liver tumors [14,15] have resulted in new drug development programs to produce novel therapeutic agents with improved toxicological profiles.

This article will review progress in developing new antiestrogens and describe the rationale for each drug design. All of the compounds under

Address correspondence to V. Craig Jordan, PhD, DSc, Professor of Cancer Pharmacology, Director, Breast Cancer Research Program, Robert H. Lurie Cancer Center, Northwestern University Medical School, 303 E. Chicago Avenue, Olson Pavilion 8258, Chicago, IL 60611.

investigation have their genesis in pharmacological investigations of tamoxifen. For convenience, the drugs have been divided into three main groups—tamoxifen analogues, derivatives of tamoxifen metabolites, or novel antiestrogens.

## TAMOXIFEN ANALOGS

A current concern with the use of tamoxifen is development of endometrial carcinoma and the potential to induce hepatocellular carcinoma. Tamoxifen is a partial estrogen agonist; it has been suggested that hydroxylation, dealkylation of the side chain, and isomerization could produce estrogenic metabolites that stimulate tumor growth [16–18]. However, this hypothesis has recently been found untenable; stable derivatives of tamoxifen that cannot isomerize after metabolic activation also provoke growth of tamoxifen-stimulated tumors under laboratory conditions [19,20]. Nevertheless, new analogs of tamoxifen that may be metabolically resistant and reduce the potential for carcinogenicity are being evaluated in clinical trials.

### Toremifene

Chlorination of the ethyl side chain of tamoxifen to produce toremifene (Fig. 1) reduces antiestrogenicity and decreases potency as an antitumor agent. However, toremifene appears to possess an advantage over tamoxifen because it has a reduced ability to induce rat liver tumors [21,22]; unlike tamoxifen, toremifene does not produce DNA adducts in the rat liver [22–24]. There is currently no evidence that tamoxifen does increase the incidence of hepatocellular carcinoma (at least above the ten-fold increased risk observed with oral contraceptives [24]). However, if hepatocarcinogenicity becomes an issue in humans, toremifene could replace tamoxifen in prevention studies. The issue of endometrial carcinoma is unresolved because there is no experience with long-term toremifene therapy. Conversely, there is no reason to believe that toremifene will not produce an identical risk for endometrial carcinoma as tamoxifen, *i.e.*, 2–3 fold [13].

Toremifene has been extensively tested for the treatment of advanced breast cancer [25–28]. The dose range is between 60–280 mg daily, but the response rate is similar to tamoxifen, *i.e.*, approximately 30% of unselected patients. Initial reports that high dose toremifene (>100 mg) will produce responses in patients with tamoxifen-resistant disease [29] are unsupported; current clinical studies demonstrate cross-resistance. A crossover study from Denmark that compared 40 mg tamoxifen daily with 240 mg toremifene daily found cross-resistance with both therapies [30]. No subsequent responses were observed at crossover. Similarly, an American study found only a 5% response in 105 patients who had failed tamoxifen but were then treated with 200 mg toremifene daily [31]. A major clinical trial of tamoxifen *versus* toremifene to treat advanced breast cancer in postmenopausal women has been completed in the United States. An analysis of the results is anticipated and FDA approval will be sought in 1995.

### Idoxifene

Hydroxylation of tamoxifen to produce 4-hydroxytamoxifen increases antiestrogenic potency [32]. However, this metabolic activation is an advantage, but not a requirement, for antiestrogenic activity. Blocking 4-hydroxylation with halogen substitutions results in compounds of weaker antiestrogenic potency [33], but does not reduce partial agonist activity. It has been reasoned that a compound with reduced 4-hydroxylation and a stable alkylaminoethoxy side chain may have less carcinogenic potential. Idoxifene (Fig. 1) is a weak antiestrogen in the rat but exhibits antitumor activity in rat mammary carcinoma models [34]. Idoxifene is resistant to metabolic degradation in laboratory tests and is detected as the principal compound in the serum of treated patients. The compound is currently undergoing Phase I/II clinical trials in England.

## DERIVATIVES OF TAMOXIFEN METABOLITES

### TAT 59

This antiestrogen is a derivative of 4-hydroxytamoxifen. Although 4-hydroxytamoxifen is a potent antiestrogen *in vitro* [35,36] and can exhibit antitumor activity in both carcinogen-induced rat mammary carcinoma models [37] and athymic mice inoculated with MCF-7 breast tumors [38], higher doses are required to produce

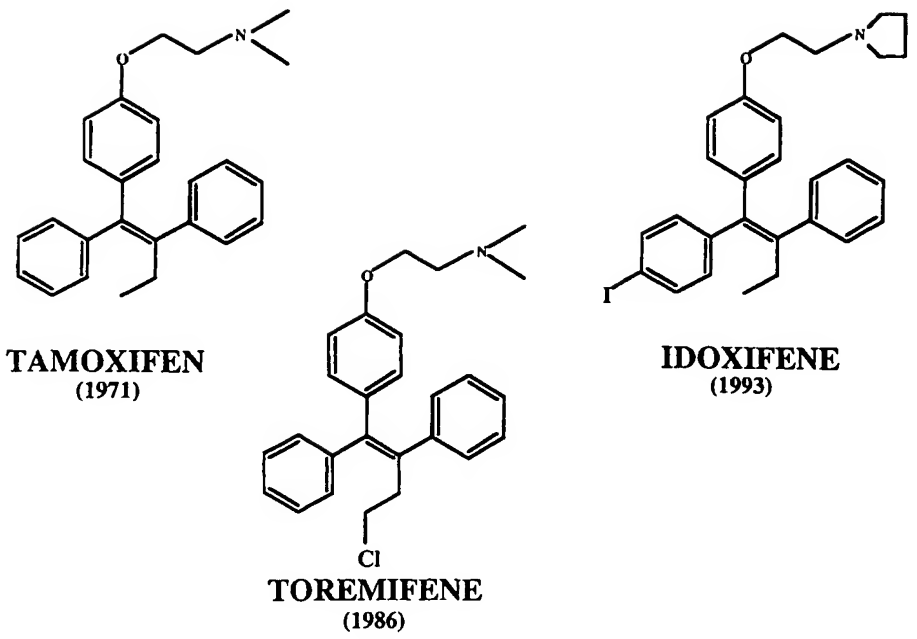


Fig. 1. Tamoxifen analogues that are in clinical trial. The date in parentheses indicate the year breast cancer studies were reported.

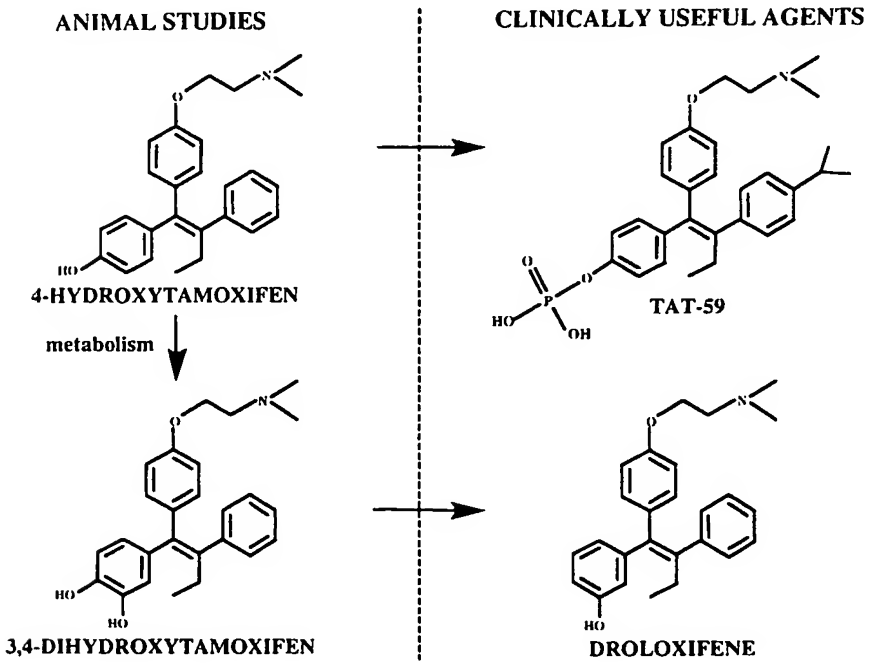


Fig. 2. The derivatives of tamoxifen that have used metabolite mimicry to design an antibreast cancer agent.

equivalent effects because the drug is vulnerable to phase II metabolism. TAT 59 is phosphorylated (Fig. 2) at the 4-hydroxy position, which could protect it from phase II metabolism, but the drug probably needs to be dephosphorylated to produce the active agent. Animal studies demonstrate antitumor activity [39]; the drug is in clinical trial in Japan.

### Droloxifene

4-Hydroxytamoxifen and 3,4-dihydroxytamoxifen are metabolites of tamoxifen [40]. Both have high binding affinity for the estrogen receptor and both exhibit antiestrogenic activity in rats [40]. Interestingly, 3,4-dihydroxytamoxifen has only weak estrogen agonist properties and is an antiestrogen in mouse uterine weight-tests [32, 40]. This contrasts with tamoxifen and 4-hydroxytamoxifen, both estrogens in mouse assays. Droloxifene (Fig. 2), the 3-hydroxylated analog of tamoxifen, has a high binding affinity for the estrogen receptor and blocks the growth of MCF-7 breast cancer cells in culture [41,42]. It does not produce DNA adducts in laboratory models of genotoxicity [23].

Droloxifene has had extensive clinical testing throughout the world. Phase I testing found few side effects [43], but as anticipated, human pharmacokinetics demonstrate a rapid excretion, with low circulating blood levels [44]. Droloxifene has been used at daily doses up to 100 mg; response rates for unselected postmenopausal patients are between 30–40% [45]. Clinical trials in postmenopausal women with advanced disease are being planned in the United States.

## A NOVEL ANTIESTROGEN: RALOXIFENE

The initial report [46] that raloxifene (originally named keoxifene) preserves bone density in laboratory animals has been confirmed [47], and studies are being set up to evaluate the worth of raloxifene as an agent to prevent osteoporosis. Raloxifene has almost complete antiestrogenic activity in high doses in the rat and mouse uterus [48] and exhibits antitumor action in the rat [49]. In contrast, it has an estrogen-like action to lower circulating cholesterol and preserves bone density in the rat [47].

Large doses will be used in clinical trials because raloxifene is rapidly cleared from the circu-

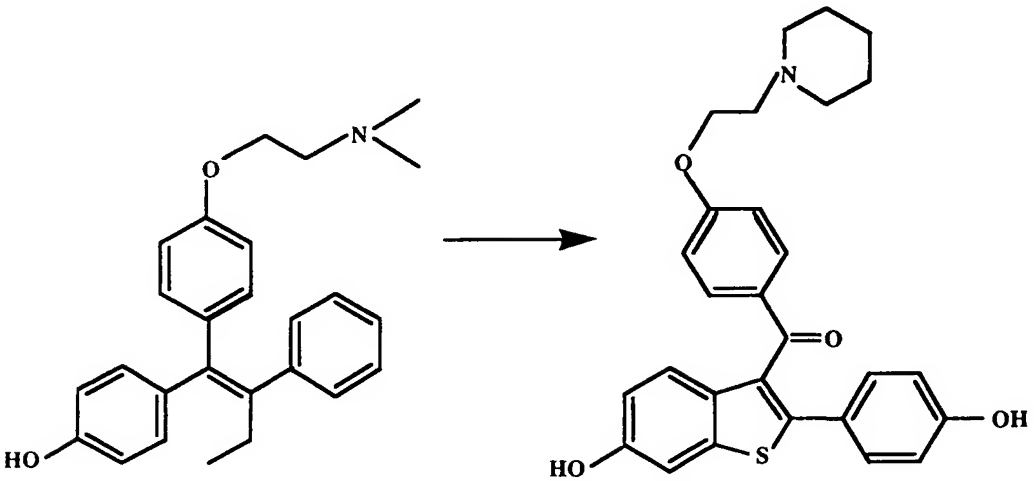


Fig. 3. A new clinical concept that is being developed to exploit the high affinity binding of antiestrogens to the estrogen receptor (ER) to produce a compound targeted to maintain bone density but block breast tumor development. Raloxifene is a high affinity antiestrogen that employs the principle first discovered with 4-hydroxytamoxifen.

lation. The hydroxyl groups make raloxifene (Fig. 3) vulnerable to phase II metabolism. Preliminary clinical studies using 200 and 600 mg raloxifene daily in several hundred postmenopausal women demonstrate that the higher daily dose will effectively lower cholesterol and reduce circulating osteocalcin levels [50].

The novel use of raloxifene opens up an exciting therapeutic opportunity. Rather than selecting women to treat with an antiestrogen to prevent breast cancer (with the added advantage of reducing their risk for osteoporosis and coronary heart disease), it is now possible to consider using safe agents to treat all postmenopausal women to prevent osteoporosis and coronary heart disease, but with the added advantage of preventing breast cancer [cf review 51]. The national impact of the new strategy on women's health may ultimately be greater than defining a narrow targeted population of women at risk only for breast cancer.

## SUMMARY

The development of tamoxifen during the past 25 years has revolutionized the treatment of breast cancer. There are now an estimated six million woman-years of experience worldwide with tamoxifen, and each year in the United States an estimated 80,000 women diagnosed with breast cancer plan to start a course of long-term tamoxifen therapy.

The clinical evaluation of tamoxifen as a breast cancer preventive in high-risk women has opened the door to new therapeutic opportunities. Pharmacological studies over the past two decades have predicted not only the value of tamoxifen as a therapeutic agent with positive effects on bones and lipids, but also predicted concerns with endometrial carcinoma and the potential for hepatocellular carcinoma. Numerous compounds have been screened, and several agents with improved toxicology are waiting for extensive clinical testing. A new range of antiestrogens with different properties and potentially different applications will soon be available to treat estrogen-regulated diseases in women.

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# Tamoxifen, Raloxifene, and the Prevention of Breast Cancer*

V. CRAIG JORDAN AND MONICA MORROW

*Departments of Molecular Pharmacology, Biological Chemistry (V.C.J.), and Surgery (M.M.), Robert H. Lurie Comprehensive Cancer Center, Northwestern University Medical School, Chicago, Illinois 60611*



## I. Introduction

IN 1896, George Beatson demonstrated that the removal of the ovaries from premenopausal women with metastatic breast cancer could, in some cases, cause regression of the disease and improve the prognosis of the patient (1). However, by 1900 Stanley Boyd had established that only one in three patients would obtain improvement for about 1 yr (2). Despite this disappointment, a link was established between an ovarian factor and the growth of some breast cancers. This observation was to become the foundation of modern clinical practice and the rationale for the use of antiestrogens to treat breast cancer (3, 4). At the turn of the century, studies were being conducted in the laboratory to complement the clinical effort. Inbred strains of mice were being established for medical research, and it was found that certain strains of mice developed a high incidence of mammary tumors. Lathrop and Loeb (5) reported that an early ovariectomy could prevent the spontaneous development of tumors but it was not until Allen and Doisey (6) identified "estrus stimulating principle" that ovarian hormones could be linked to the development of breast cancer. By 1936, Professor Antoine Lacassagne, again working with high-incidence strains of mice, suggested that if breast cancer was caused by a special hereditary sensitivity to estrogen, then the disease could be prevented by developing a therapeutic antagonist to estrogen action in the breast (7). However, there were no therapeutic antagonists of estrogen at that time, nor was there a target to design drug molecules. Nevertheless, exciting developments in the discovery of nonsteroidal estrogens would establish the structural basis of carrier molecules, which resulted in the design of the two drugs, tamoxifen and raloxifene (Fig. 1), both originally described as antiestrogens and used today in a clinical trial to prevent breast cancer in high-risk women (see *Section IX*).

Estrogen action in the 1930s was assayed using ovariectomized mice as originally described by Allen and Doisy (6). Using this technique, parallel research ventures resulted in the discovery of the triphenylethylene-based estrogens (8–10) and the stilbene-based estrogens (Fig. 1). The triphenylethylenes are long acting and are stored in body fat (11–14), whereas the hydroxystilbene derivatives are short acting (9, 15, 16), primarily because of rapid-phase II metabolism after absorption. However, Dodds and associates (17, 18) described an extremely potent compound, diethylstilbestrol (Fig. 1), that was widely used in gynecology and also subsequently used, at high doses, as a treatment for advanced breast cancer in postmenopausal women (19, 20).

In the 1950s and 1960s it became clear that adrenalectomy, with glucocorticoid support, could also improve the prognosis of some postmenopausal women with advanced breast

Address reprint requests to: V. Craig Jordan, Ph.D., D.Sc., Director, Lynn Sage Breast Cancer Research Program, Robert H. Lurie Comprehensive Cancer Center, Olson Pavilion 8258, 303 E. Chicago Avenue, Chicago, Illinois 60611 USA.

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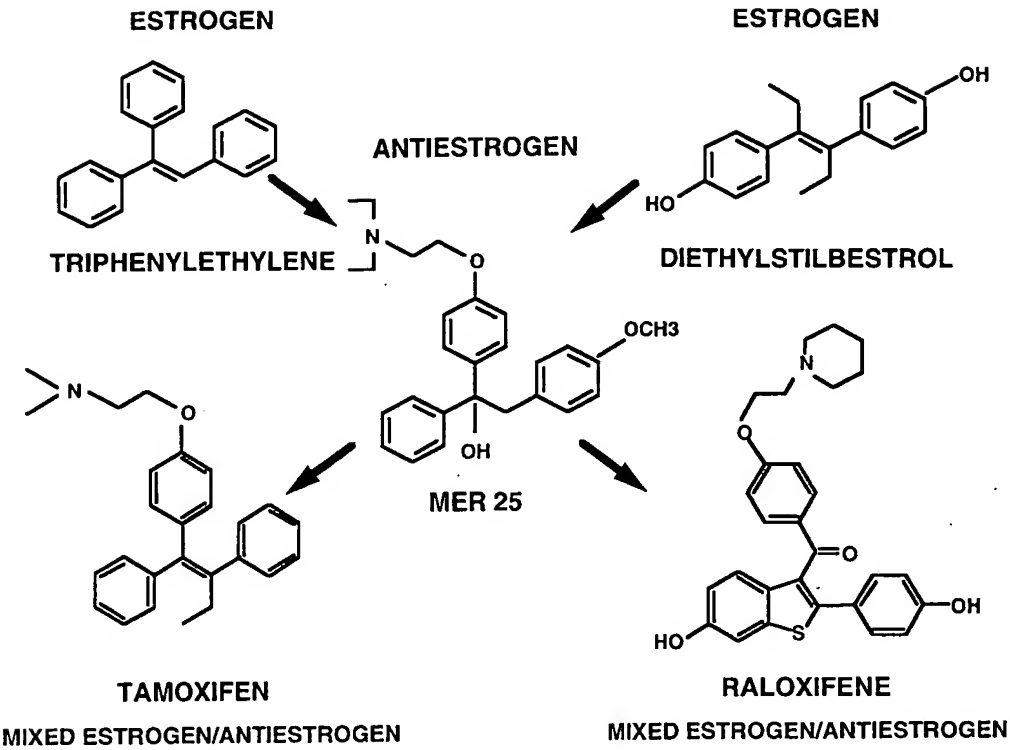


FIG. 1. The discovery of MER-25, and the knowledge that a strategically placed alkylaminoethoxy side chain confers antiestrogenic properties, was important to develop the antiestrogens tamoxifen and raloxifene from the known nonsteroidal estrogens, triphenylethylene and diethylstilbestrol. Tamoxifen is used for the endocrine treatment of all stages of breast cancer and is available for the reduction of breast cancer incidence in high-risk women. Raloxifene is used for the prevention of osteoporosis in postmenopausal women, but because a preliminary evaluation shows a reduction in the risk of breast cancer, raloxifene is to be evaluated for the prevention of breast cancer in high-risk postmenopausal women. The clinical trial STAR started to recruit the 22,000 volunteers in 1999.

cancer (21). In fact, about one third of the women responded, *i.e.*, about the same proportion as premenopausal women after oophorectomy. The reason for the apparently arbitrary responses, however, would not become clear until the discovery of the estrogen receptor (ER) by Jensen and Jacobson (22) and the subsequent application of this knowledge to predict the hormone responsiveness of a patient's tumor to endocrine ablation (23). This was an extremely important finding because it prevented those patients who had an ER-negative tumor from having additional major surgery with little hope of a response. Only patients whose tumors had high levels of ER were likely to respond to endocrine ablative surgery (24). It is important, however, to stress that in the early 1970s, there was no significant clinical experience available with the class of drugs called antiestrogens, and no large clinical studies had linked the efficacy of antiestrogens with the presence or absence of the ER. Several antiestrogens had been tested in small clinical studies, but tamoxifen, the first clinically useful antiestrogen for the treatment of advanced breast cancer in postmenopausal women, was not approved by the Food and Drug Administration in the United States until 1977 (25).

Although antiestrogens are important therapeutic agents today, 40 yr ago there was very little interest in treating breast cancer with new hormonal drugs, and most of the research in endocrinology was focused on an understanding of reproduction. The discovery of the nonsteroidal antiestrogens was serendipitous and resulted from an interest in contraception in the 1950s. The first nonsteroidal antiestrogen to be reported in the literature, MER25 (Fig. 1), was described by Lerner and co-workers in 1958 (26) as an agent that had no other hormonal or antihormonal properties in any species tested. In fact, it was a blocking drug for estrogen action with almost no estrogenic properties. The drug failed in clinical trial because large doses were required (MER25 has low potency), which caused serious central nervous system side effects (27). On one hand this was disappointing but, it must be stressed, that a pure antiestrogen such as MER25 would ultimately have been catastrophic as an agent to prevent breast cancer. Drug discovery switched to the triphenylethylene-based compounds that resulted first in clomiphene and then tamoxifen (25). Subsequently, drug discovery concentrated on compounds with a high affinity for ER (25). Only a research focus on cancer in the 1970s facilitated tamoxifen's development as a breast cancer therapy for all stages of the disease (25).

The critical property of the so called "antiestrogens," which permitted their subsequent development as long-term preventives for breast cancer, was that they are antiestrogens at some sites, like the breast, but had estrogen-like properties at other sites to maintain bone density and lower circulating cholesterol (28–35). The unusual target site-specific action as an estrogen or as an antiestrogen was true for both tamoxifen and raloxifene (29, 30). Lacassagne's prediction (7) of developing an antagonist to estrogen action to prevent breast cancer in healthy women would not have occurred if the

available drugs had increased the risk of osteoporosis and coronary heart disease.

The goal of this review is to provide an up-to-date analysis of the current status of efforts to prevent breast cancer in women by the strategic use of antiestrogens. One aim of our review is to identify the principles, established in the laboratory, that have, through the clinical trial process, proven to be valid in patients with breast cancer or women at risk for breast cancer. The review will also provide the scientific basis for the ongoing trial called Study of Tamoxifen And Raloxifene (STAR). This trial is examining the worth of raloxifene, a drug approved for the prevention of osteoporosis, to prevent breast cancer in postmenopausal women with elevated risk factors. The biological basis for consideration of the antiestrogen raloxifene to be used as a breast cancer preventive is described in *Section VIII*. In the interests of space it is not possible to review the antiestrogen literature exhaustively, but we intend to provide sufficient background to link laboratory research with clinical results.

## II. Lacassagne's Prevention Principle: A Target and an Estrogen Antagonist

In 1962, Jensen and Jacobson (22) demonstrated that [$^3$H]estradiol bound to and was retained by estrogen target tissue, *e.g.*, uterus, vagina, and pituitary gland, in the immature female rat. By contrast, tissues that did not respond to estrogen did not retain [$^3$H]estradiol. Jensen proposed that an ER must be present in estrogen target tissues to capture circulating steroids and initiate the cascade of biochemical events associated with estrogen action in that particular tissue. Gorski and co-workers (36, 37) first identified the ER as an extractable protein from rat uterus. Subsequently, the groups of Gorski *et al.* (38) and Jensen *et al.* (39) independently proposed subcellular models of estrogen action in target tissues. However, Jensen and associates (23) took the process one step further by proposing the clinical ER assay to predict hormone-responsive breast cancer. Thus, a mechanistic link between estrogen action and the growth of breast cancer was established.

The MCF-7 breast cancer cell line is ER positive (40, 41), and the cells have found ubiquitous applications in cancer research laboratories throughout the world (42). Most importantly, access to these cells has resulted in a fundamental change in the understanding of hormone action that has resulted in the discovery of the steroid receptor superfamily of receptors (43, 44). Jensen and co-workers (45, 46) first developed monoclonal antibodies to ER derived from MCF-7 cells. The antibodies were used to establish that the ER was a nuclear protein (47), and the technology of immunocytochemistry is now standard for the determination of receptor status in breast biopsies (48, 49). However, the application of monoclonal antibodies as probes to clone and sequence the ER gene (50–52) is of fundamental significance for the understanding of the ER as a nuclear transcription factor.

The ER is a nuclear protein (47, 53, 54), which, for convenience, is subdivided into six functional domains (Fig. 2) (55, 56). The E regions make up the steroid-binding domain that undergoes a conformation change to lock estradiol into its hydrophobic pocket (see *Section IV.A*). Changes in conformation of the ER permits the binding of coactivators to the activating function 1 and 2 regions (AF-1 and 2) (57–59) and facilitates the interaction of the ER with DNA through the DNA-binding domain (C region) (60, 61). The transcription unit is selectively located as a homodimer in the promoter region of estrogen-responsive genes to initiate the events associated with estrogen-stimulated cell replication. Unfortunately, it is not possible to provide much more than the basic concepts in hormone action because our article is focused on progress in breast cancer chemoprevention. The topic of gene regulation is a rapidly evolving story, so we strongly recommend that interested readers consult recently published reviews that provide further information (62–64).

The second facet of Lacassagne's hypothesis is the requirement for an antiestrogen to block estrogen action. Tamoxifen blocks the binding of [$^3$H]estradiol to the ER derived from rat uterus (65–68) or human tumor (69, 70). However, initial clinical studies with tamoxifen were conducted exclusively on unselected populations of postmenopausal women with advanced breast cancer (3, 4), and not until 1977 was it noted that tamoxifen was more likely to be effective in ER-positive breast cancer (71). Tamoxifen is currently used as a palliative therapy in the treatment of pre- and postmenopausal patients with ER-positive advanced (Stage IV) breast cancer. By contrast, the application of the concept of adjuvant therapy has revolutionized the treatment of breast cancer. Systemic adjuvant therapy is used after breast surgery to destroy undetected micrometastases around a woman's body.

Adjuvant studies with tamoxifen have proved to be successful in increasing survival (72–74) but, perhaps most importantly, the interaction between laboratory and clinical research endeavors has ultimately elucidated both the principal mechanism of action of tamoxifen as an antitumor agent in women and identified those women most likely to benefit from adjuvant tamoxifen treatment. During the past 12 yr there has been some confusion in the literature about whether tamoxifen was active in ER-positive breast cancer exclusively or whether ER-negative breast cancer could respond (75–77). Additionally, there was controversy as to whether tamoxifen was significantly active as an adjuvant in premenopausal women (78). The reader is referred to recent reviews on the clinical investigation and development of tamoxifen (79–83) for further information, but we will summarize the latest findings of the world-wide randomized clinical trials (84). It is important to appreciate that the general principles derived from the use of tamoxifen as a therapy for breast cancer can be used as a basis for consideration of tamoxifen as a estrogen antagonist for the prevention of breast cancer.

## III. Tamoxifen as an Antitumor Agent

The 1998 Oxford Overview Analysis (84) involved any randomized trial that began before 1990. The analysis included 55 trials of adjuvant tamoxifen *vs.* no tamoxifen before recurrence. The study population comprised 37,000 women with node-positive and node-negative breast cancer, thus comprising 87% of world evidence of known randomized

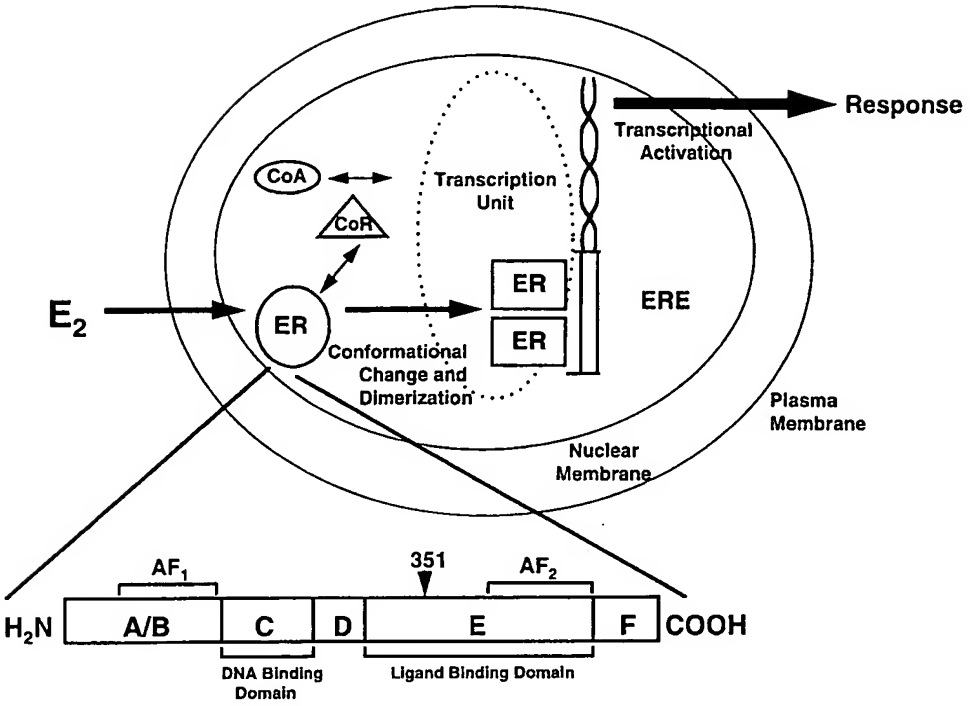


FIG. 2. A subcellular model of estradiol ($E_2$) action in a target tissue. Estradiol diffuses into all cells but binds to the ER specifically located in estrogen target tissues (*e.g.*, uterus, vagina, some breast cancers, etc.). The steroid receptor complex undergoes a conformational change and dimerizes before binding to an estrogen response element (ERE) in the promotor region of an estrogen-responsive gene. A transcription unit is formed by interaction with coactivator (CoA) molecules to initiate RNA synthesis and ultimately the estrogen-stimulated cellular response. Corepressor (CoR) modules are believed to prevent transcription by exclusively interacting with antiestrogen ER complexes. The ER is divided into six regions (A–F). The DNA-binding domain (C) is essential for the interaction of the ER with the ERE. The ligand-binding domain (E) is the site of $E_2$ binding and the site of competitive binding by antiestrogens. The AA351 is identified in the E region because it is the known site of an interaction with the alkylaminoethoxy side chain of raloxifene. The activating function (AF-1 and -2) regions are the areas of the ER that interact with coactivators to form the transcription unit at an estrogen-responsive gene.

clinical trials. Of these women, fewer than 8,000 had a very low or zero level of ER and 18,000 were classified as ER positive. The ER status of the remaining nearly 12,000 women was unknown, but based on the normal distribution of ER in random populations, the authors estimated that two-thirds would be ER positive.

This clinical trial data base (84) can now be used to answer the questions raised over the past two decades by laboratory results and hypotheses. In the 1970s three laboratory observations emerged that merited evaluation in clinical trial: 1) tamoxifen blocks estrogen binding to the ER, making patients with ER-positive disease more likely to respond than those with ER-negative disease (85), 2) tamoxifen prevents mammary cancer in rats (86, 87) so the drug could reduce the incidence of primary breast cancer, and 3) long-term treatment was better than short-term treatment to prevent rat mammary carcinogenesis; therefore, longer adjuvant therapy with tamoxifen should be superior to short-term adjuvant therapy (88–90), *i.e.*, 5 yr of tamoxifen should be superior to 1 yr of tamoxifen. By the late 1980s, tamoxifen had been shown in the laboratory to block estrogen-stimulated breast tumor growth but to encourage the growth of human endometrial cancer implanted in the same athymic mouse (91, 92). The clinical question therefore became "are patients, who are receiving long-term adjuvant tamoxifen therapy, at risk for an increased incidence of endometrial cancer?" (92).

The process of evaluating the impact of translational research is important to establish what works and achieves clinical progress and what does not. A clinical trial should not begin without a strong hypothesis and the incorporation of relevant scientific results. For convenience, the discussion in this section will be subdivided, but the end points of duration of tamoxifen usage, menopausal status, and ER status interact, making the size of a pharmacological effect subject to change.

### *A. ER status and the duration of tamoxifen*

The ER status of the patient is highly predictive of a treatment response to long-term tamoxifen therapy. The treatment effect, based on receptor status, is summarized in Table 1. The recurrence reductions produced by tamoxifen in ER-positive patients are all highly significant ($2P < 0.00001$), and the trend between the different durations of tamoxifen is also highly significant ($\chi^2 = 45.5$, $2P < 0.00001$). By contrast, the therapeutic effect of tamoxifen on ER-negative patients is minimal. Additionally, the questions could be asked, "Does more ER give a better response to tamoxifen?" and "Does an additional progesterone receptor (PgR) assay help to improve the results with tamoxifen?" In the trials of about 5 yr of tamoxifen treatment, the proportional reductions of recurrence were 43 ± 5% and 60 ± 6% for patients with below or above 100 fmol/mg cytosol ER protein. This translated to a reduction in mortality of 23 ± 6% and 36 ± 7%, respectively.

TABLE 1. A comparison of the proportional risk reduction of adjuvant tamoxifen therapy based on ER status

| | Duration of tamoxifen therapy (yr) | | |
|---|---|---|---|
| | 1 | 2 | 5 |
| a.) ER-poor | | | |
| % Reduction in recurrence rates (±SD) | 6 ± 8 | 13 ± 5 | 6 ± 11 |
| % reduction in death rates (±SD) | 6 ± 8 | 7 ± 5 | −3 ± 11 |
| | Duration of tamoxifen therapy (yr) | | |
| | 1 | 2 | 5 |
| b.) ER-positive | | | |
| % Reduction in recurrence rates (±SD) | 21 ± 5 | 28 ± 3 | 50 ± 4 |
| % Reduction in death rates (±SD) | 14 ± 5 | 18 ± 4 | 28 ± 5 |

Nearly 8,000 patients are ER poor and 18,000 patients are ER positive. [Derived from Ref. 84].

Clearly, one can conclude the ER is a powerful predictor of tamoxifen response, a conclusion consistent with tamoxifen's proven mechanism of action as an estrogen antagonist in breast cancer (82). Although PgR-positive status might be thought to be of benefit, these data show that there was little additional value if the tumor was already ER positive. A comparison of interactions is shown in Table 2. A comparison of the 2,000 women who had ER-positive and PgR-negative tumors and the 7,000 women who had ER-positive and PgR-positive tumors shows there was no apparent difference in the effect of tamoxifen on either the recurrence rates or mortality rates. Additionally, the numbers were too few (602 women) in the Overview Analysis (84) to allow a meaningful prediction of the benefits of tamoxifen in patients who had an ER-negative but PgR-positive tumor.

The Overview Analysis also provides unequivocal proof of the laboratory principle (88–90) that longer adjuvant tamoxifen therapy was predicted to provide more benefit. The duration of therapy is extremely important for the ER-positive premenopausal woman with large amounts of circulating estrogen that can rapidly reverse the effect of short-term tamoxifen treatment. The effect of the duration of tamoxifen treatment on the reduction of recurrence rates and the reduction of death rates is shown in Fig. 3. The duration of tamoxifen therapy is critical for the premenopausal patient: the effect of 1 yr of treatment is virtually nonexistent compared with the benefit of 5 yr of treatment. It is also important to point out that the reduction of death rates in women under 50 yr of age and over 60 yr of age treated with 5 yr of tamoxifen is identical, at around 33% (Table 3). By

TABLE 2. A comparison of the proportional risk reduction of adjuvant tamoxifen therapy based on PgR status in populations of ER-positive patients

| | ER + PgR− (n = 2000) | ER + PgR+ (n = 7000) |
|---|---|---|
| % Reduction in recurrence rates (±SD) | 32 ± 6 | 37 ± 3 |
| % Reduction in death rates (±SD) | 18 ± 7 | 16 ± 4 |

[Derived from Ref. 84.]

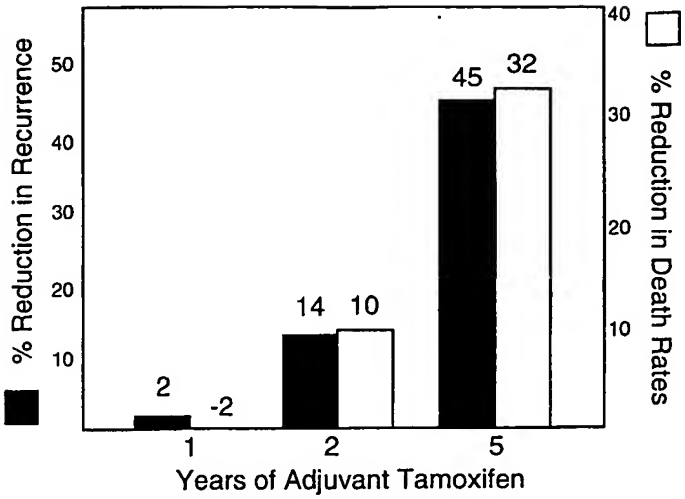


FIG. 3. The relationship between the duration of adjuvant tamoxifen therapy in ER-positive premenopausal patients and the reduction in recurrence and death rate. A longer duration of treatment has a dramatic effective on patient survival. [Derived from Ref. 84.]

TABLE 3. Proportional risk reductions in 60- to 69-yr-old breast cancer patients when the known ER-poor patients are excluded

| | Duration of tamoxifen therapy (yr) | | |
|---|---|---|---|
| | 1 | 2 | 5 |
| % Reduction in recurrence rates (±SD) | 26 ± 6 | 33 ± 4 | 54 ± 5 |
| % Reduction in death rates (±SD) | 12 ± 6 | 12 ± 5 | 33 ± 6 |

The duration of adjuvant tamoxifen is 1, 2, or 5 yr [Derived from Ref. 84].

contrast, the effect of tamoxifen duration on women over the age of 60 is less dramatic because 1 yr of tamoxifen is much more effective in postmenopausal women. These data are illustrated in Table 3, which shows a 2- to 3-fold increase in the effectiveness of tamoxifen when the duration is increased from 1 to 5 yr, whereas there is a 20-fold increase in tamoxifen's effectiveness for premenopausal women with an increased duration of 1–5 yr (Fig. 3).

### *B. Contralateral breast cancer*

Tamoxifen consistently reduces the risk of contralateral breast cancer (*i.e.*, a second primary breast cancer in the other breast) independent of age (84). Women have a proportional risk reduction that is 27 ± 11% or 31 ± 7% if they are below or above the age of 50, respectively. The principle "longer is better" is also true for the reduction of risk for contralateral breast cancer with adjuvant tamoxifen therapy. Five years is better then 2 yr or 1 yr of adjuvant therapy with tamoxifen (Fig. 4). In fact, 1 yr of adjuvant tamoxifen does not significantly reduce the incidence of contralateral breast cancer compared with control because the SD is so large (13 ± 13% reduction compared with control).

It is interesting to note that a quarter of the women allocated to the known adjuvant trials in the Overview Analysis (84) were Japanese, who have an annual incidence of con-

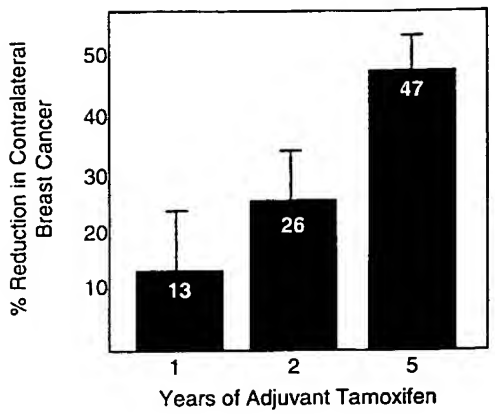


FIG. 4. The relationship between the duration of adjuvant tamoxifen and the reduction in contralateral breast cancer. A longer duration is clearly superior, and the 5-yr result that produces a 47% reduction in contralateral breast cancer is equivalent to the result observed in the tamoxifen prevention trial presented in Fig. 9. [Derived from Ref. 84.]

tralateral breast cancer in patients not receiving tamoxifen of 2 per 1,000 compared with 6 per 1,000 elsewhere in the world. Therefore, if 5 yr of tamoxifen therapy can halve contralateral breast cancer, then the absolute benefit for Japanese women would be 1 per 1,000 and 3 per 1,000 elsewhere for both young and old women. Finally, the proportional reduction in contralateral breast cancer appears to be similar in women whose initial tumor being treated with tamoxifen was ER-poor (29 ± 15%) compared with the rest of the study population (30 ± 6%). This is an important result for the potential application of tamoxifen for the reduction of contralateral breast cancer in the woman with a primary breast cancer that is unequivocally ER negative.

### C. Endometrial cancer

The overall increase in the incidence of endometrial cancer in the Overview Analysis was 2- to 3-fold (84). There was no association with dose, *i.e.*, 20 mg and 30–40 mg daily produced relative risk (RR) ratios of 2.7 and 2.4, respectively. However, there was a suggestion that 1 and 2 yr of tamoxifen doubled the incidence of endometrial cancer and 5 yr quadrupled the incidence. However, the side effect is so rare (*i.e.*, the numbers are too small) that the risk ratios are not significantly different from one another for each duration of tamoxifen. It is important, however, to state that the absolute increase in endometrial cancer was only half as big as the absolute decrease in contralateral breast cancer.

The Overview Analysis was able to identify 3,673 women who took 5 yr of adjuvant tamoxifen. With 26,400 woman years of follow-up before breast cancer recurrence in this group, there were seven endometrial cancer deaths. It is estimated that during the whole first decade, the cumulative risk was two deaths per 1,000 women. It is important to state that the current knowledge about the association of tamoxifen with endometrial cancer will improve these statistics. In general, the reported trials were conducted without awareness of the endometrial side effects of tamoxifen. This is no longer the situation, and early detection will improve mortality figures associated with tamoxifen.

### D. Conclusions

Tamoxifen has been extensively tested in clinical trials of adjuvant therapy for 20 yr. The Overview shows that the proportional mortality reductions were similar for women with node-positive or node-negative disease (84). However, the absolute reductions in mortality were much greater in node-positive than node-negative disease. Additionally, patients with ER-positive disease have an increased reduction in death rate with longer duration of tamoxifen treatment, whereas patients who are ER-negative do not benefit from tamoxifen, regardless of the duration of therapy. The value of a long duration of treatment is most important for the premenopausal patient (Fig. 3). This latter finding is new, as the results for premenopausal women could not be ascertained with certainty in earlier overviews (74). The Oxford Overview Analysis has established the veracity of the laboratory concepts that tamoxifen would be most effective in ER-positive disease, longer duration would be more beneficial, and tamoxifen would prevent primary breast cancer, in this case contralateral disease (85–90).

Overall, the absolute improvement in recurrence was greater during the first 5 yr after surgery, but improvement in survival increased steadily throughout the first 10 yr. This is an important finding because the patient is clearly benefiting from tamoxifen even after therapy has been discontinued. There is an accumulation of the tumoristatic/tumoricidal actions of tamoxifen for at least the first 5 yr of treatment, but the benefit continues after therapy stops. This is also true for the reduction in contralateral breast cancer; the breast seems to be protected so the value remains after therapy stops. This observation is extremely important for the application of tamoxifen as a preventive because a 5-yr course of tamoxifen would be expected to protect a woman from breast cancer for many years afterward.

Finally, the risk/benefit ratio of tamoxifen therapy can be stated to be strongly in the benefit category. The risk of endometrial cancer, a concept derived from laboratory studies (92), is of concern, but the benefits clearly outweigh the risks. In contrast, early concerns about the carcinogenic effects of tamoxifen in the rat liver (see *Section VII*) do not translate to the clinic as there is no evidence from the Overview Analysis of an increase in either liver or colorectal cancer in patients who take tamoxifen (84)

## IV. Selective ER Modulation

Nonsteroidal antiestrogens were originally defined as compounds that would inhibit estradiol-stimulated rat uterine weight. The compounds tamoxifen (ICI 46,474) (93), nafoxidine (U, 11,100A) (94), nitromifene (CI628) (95), and clomiphene (MRL 41)(96) are all partial estrogen agonists in the uterus that also inhibit dimethylbenzanthracene (DMBA)-induced rat mammary tumor growth (67, 97, 98) and the growth of ER-positive MCF-7 breast cancer cell growth *in vitro* (99). Thus, in the 1960s and 1970s, antiestrogenicity was correlated with antitumor activity. However, the finding that the compounds expressed increased estrogenic properties, *i.e.*, vaginal cornification and increased uterine weight in the mouse (93, 100), raised questions about

the reasons for the species specificity. One obvious possibility was species-specific metabolism, *i.e.*, the mouse converts antiestrogens to estrogens via novel metabolic pathways. However, no species-specific metabolic routes to known estrogens (101, 102) have been identified, but knowledge of the mouse model created a new dimension for study that ultimately led to the recognition of the target site-specific actions of antiestrogens. This concept was subsequently referred to as selective ER modulation (SERM) to describe the target site-specific effects of raloxifene, an antiestrogen originally targeted for an application in breast cancer but now used, paradioxically, as a preventive for osteoporosis (see *Section VII*). Now the whole class of drugs are known as SERMs.

The ER-positive breast cancer cell line MCF-7 (for a review see Ref. 42) can be heterotransplanted into immune-deficient athymic mice but the cells will only grow into tumors with estrogen support. Paradoxically, tamoxifen, an estrogen in the mouse, does not support tumor growth (103) but stimulates mouse uterine growth with the same spectrum of tamoxifen metabolites present in both the uterus and the human tumor (28). To explain the selective actions of tamoxifen in different targets of the same host, it was suggested that the ER complex could be interpreted as a stimulatory or inhibitory signal at different sites (28). The concept was consolidated with experimental evidence from two further models. First, tamoxifen and raloxifene maintain bone density in the ovariectomized rat, but both compounds inhibit estradiol-stimulated uterine weight (29) and prevent carcinogen-induced mammary tumorigenesis (30). Second, the finding that tamoxifen would partially stimulate the growth of a human endometrial carcinoma transplanted into athymic mice (91) allowed the investigation of two human tumors bitransplanted in the same mouse to determine whether tamoxifen could inhibit estrogen-stimulated growth of two tumors in the same host equally (92). Tamoxifen demonstrated target site specificity: breast tumor growth was controlled but endometrial tumors continued to grow. Again the range of tamoxifen metabolites were consistent in all target tissues despite the contrasting biological responses, so it was concluded that the ER complexes must be interpreted differently in different target tissues.

During the past decade an intense effort has been made to discover the reason for the target site-specific effects of antiestrogens. Not only will this knowledge permit a rational application of tamoxifen and raloxifene in patients, but also the discovery of new mechanisms for drug selectively will open the door for new innovations in drug discovery. For the sake of completeness, we will briefly consider some of the current hypotheses that could explain the molecular mechanisms of antiestrogen action in different tissue sites.

### *A. Antiestrogenic activity at the ER*

The crystallization of the ligand-binding domain of the ER with estradiol and raloxifene has provided an important insight into the conformational changes that occur in the receptor (104) liganded with an estrogen or an antiestrogen, respectively. Estradiol causes helix 12 to seal the ligand inside the hydrophobic pocket of the ligand-binding domain (Fig. 5A). This causes receptor activation through the binding

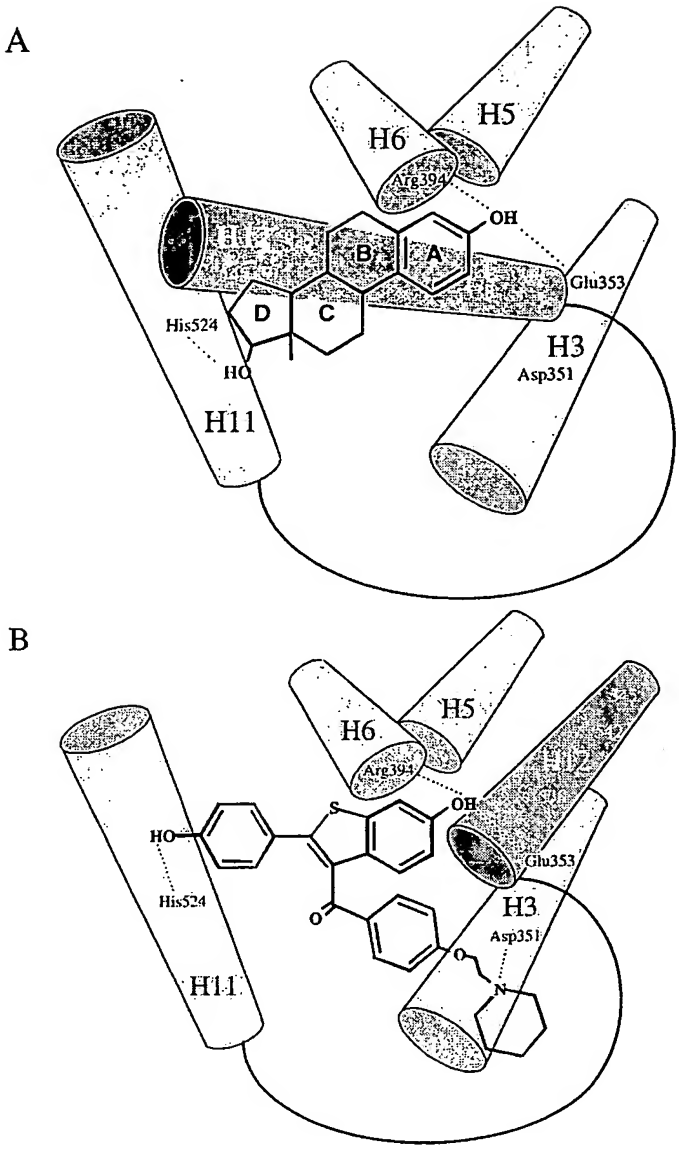


FIG. 5. Comparison of the binding of estradiol (diagram A) and raloxifene (diagram B) in the ligand-binding domain of the human ER. The key event is the repositioning of helix 12 to seal the steroid in the hydrophobic pocket. This event allows the ER complex to recruit coactivators for the transcription complex. The side chain of raloxifene prevents recruitment of coactivators by first masking AA 351 in helix 3, which is critical for the relocation of helix 12. Coactivators cannot bind to the AF2 region of helix 12 because it cannot seal the ligand-binding domain. [Derived from Ref. 104.]

of coactivators on the surface of helix 12 (see *Section IV.B*). By contrast, the binding of raloxifene prevents helix 12 from sealing the hydrophobic pocket (Fig. 5B), and gene transcription cannot occur because coactivators cannot bind. Unfortunately, the final shape of the ER and anti-ER complexes do not tell us how the tertiary changes in protein structure occur. However, the crystal structure provides proof of the critical importance of AA351 (aspartate) for raloxifene action. The alkylaminoethoxy side chain is the essential structural feature of nonsteroidal antiestrogens (for a review see Ref. 105). The distance between the nitrogen and the oxygen must be optimal (106), the conformations available to the side chain

must not be restricted (107), and the basicity of the nitrogen must be correct (108). Removal of the side chain results in loss of all activity or an increase in estrogenic properties (109). The side chain was originally predicted (110, 111) to bind to an "antiestrogenic region" in the ligand-binding domain of the ER to neutralize the estrogenic properties of the receptor. Simply stated, the antiestrogen was perceived to act like a stick to prevent the jaws of the ER from closing around the ligand. Looked at another way, an estrogenic complex would only be created by the protein enveloping the ligand. For the nonsteroidal antiestrogens, the "antiestrogenic region" is now known to be AA351 on helix 3. The discovery of an ER mutant in a tamoxifen-stimulated MCF-7 breast tumor (112) and the finding that it can increase the estrogenic properties of 4-hydroxytamoxifen (113, 114), the active metabolite of tamoxifen (115), and convert raloxifene from an antiestrogen to an estrogen (116, 117) is valuable biological proof that AA 351 is important for the antiestrogenic activity of these specific compounds. This interaction, at the critical contact point of helix 3 and helix 12, prevents helix 12 from sealing the ligand into the binding pocket (Fig. 5B). Interestingly, a recent report of the crystal structure of 4-hydroxytamoxifen and the ligand-binding domain of ER (118) shows a complex interaction of the side chain with several amino acids including AA 351. The distance between AA 351 and the nitrogen of 4-hydroxytamoxifen is further than the comparable interaction in raloxifene. This difference between the crystal structure of the raloxifene ER complex (104) and the 4-hydroxytamoxifen ER complex (118) may explain the promiscuous nature of the 4-hydroxytamoxifen ER complex. However, it must be stressed the AA 351 has no role for the antiestrogenic action of the pure antiestrogen ICI 182,780 (117) and other amino acids may be found to be involved in the antiestrogenic mechanisms of novel compounds in the future.

Nevertheless, this two-dimensional model, which describes antiestrogen and estrogen action, is too simple to encompass all the complexities of the target site-specific actions of the antiestrogens. Furthermore, the crystallization data do not include the conformational information about the other half (*i.e.*, the A, B, C, or F domains illustrated in Fig. 2) of the ER that control interaction with transcription factors and binding to DNA. Subtle changes in the whole protein shape could be responsible for changes in the intrinsic efficacy of the receptor complex at different target sites.

### *B. Coactivators for ER*

A host of coactivator and corepressor proteins has been implicated in the construction of a transcription complex in target cells (63, 64, 119–123). The finding that an antiestrogen ER complex could become increasingly estrogenic in different cell contexts (124, 125) raised the possibility that the differential distribution of coactivators or corepressors could be responsible for changes in estrogenicity between the breast and, for example, bones (126, 127). The activating function (AF-2) region in the ligand-binding domain (Fig. 2) is known to be repressed by tamoxifen and raloxifene, but the AF-1 region is unaffected by tamoxifen binding (125). Clearly, the shape of a particular complex of ligand and ER will be different for different drugs (125). Thus coactivators could modulate estrogenicity differentially in different target sites. The candidate proteins could therefore amplify the anti-ER complex into an estrogenic complex. Alternatively, the anti-ER complex might recruit completely new proteins at a specific target site to induce or to suppress gene transcription.

### *C. Alternate response elements on DNA*

Anti-ER complexes can bind to an estrogen response element but cannot recruit coactivators to initiate transcription of estrogen-responsive genes (114). However, it is possible that the anti-ER complex can bind to alternate sites in the promoter region to initiate transcription. Alu DNA repeats were originally thought to be functionally inert, but these elements may be able to activate gene transcription with antiestrogens through an ER-related mechanism (128, 129). Additionally, a specific region of the transforming growth factor-$\beta$ promoter is believed to be activated by raloxifene (130). A raloxifene response element has been identified (131), but the authors are now convinced that a simple protein DNA interaction does not occur (132).

### *D. An alternate ER-ER$\beta$*

The discovery of a second ER, named ER$\beta$ (133), has introduced a new dimension into the possible mechanisms of tamoxifen or raloxifene action. Although ER$\beta$ has similar functional homology in the DNA-binding domain, there is only 55% homology between ER$\beta$ and ER$\alpha$ in the ligand-binding domain. Clearly, one possibility to explain the target site specificity and altered estrogenicity of antiestrogens is a differential distribution of ER$\alpha$ and -$\beta$ to different tissues (134). The mechanism of action of the differential pharmacology between ER$\alpha$ and -$\beta$ may also involve different methods of gene activation. A novel signal transduction pathway has been identified as a protein-protein interaction between ER$\beta$ anti-ER complexes and AP-1 (fos and jun) (135) that is capable of activating a reporter gene. Estradiol, however, does not activate the reporter. Therefore, the pathway would be of pharmacological rather than physiological significance. Interestingly, an ER$\alpha$ tamoxifen complex will activate AP-1 reporter systems in the context of an endometrial cancer cell (136). This has led to speculation that the target site specificity of antiestrogens could be both receptor and context selective.

## V. Biological Basis for Tamoxifen as a Breast Cancer Preventive

With the molecular mechanisms of antiestrogens as a background, it is now appropriate to consider the scientific rationale for selecting tamoxifen to be tested as a breast cancer preventive, based on its pharmacological properties. Knowledge converged over the past 25 yr to make the choice of testing tamoxifen in well women a logical extension of clinical experience. Tamoxifen was selected for testing as a preventive based on 1) animal studies that demonstrated it could prevent carcinogenesis, 2) an extensive clinical experience

that showed few serious side effects, 3) a beneficial profile of estrogen-like action in maintaining bone density, and 4) tamoxifen reduces circulating cholesterol. The fact that tamoxifen was already known to reduce the incidence of contralateral breast cancer made the drug the primary agent to test in high-risk women. The pharmacological properties of tamoxifen have been recently reviewed extensively (82); therefore, the purpose of this section is to act as a framework for a comparison with raloxifene in *Section VIII* and as a prelude to considering the current STAR trial (*Section IX*).

### *A. Animal models*

Tamoxifen prevents rat mammary carcinogenesis induced by dimethylbenzanthracene, *N*-nitrosomethylurea, and ionizing radiation (86–89, 30, 137), and long-term treatment prevents spontaneous carcinogenesis in C3H/OUJ mice infected with mouse mammary tumor virus (138). The latter result is of interest because tamoxifen is classified as an estrogen in the uterus and vagina of the mouse (93, 100). This, again, illustrates the target site specificity of tamoxifen in the mouse model, as mammary cancer is prevented almost completely. Although the athymic mouse model heterotransplanted with breast cancer cell lines has been extremely instructive for the use of therapeutic tamoxifen (103), and valuable as a model for understanding drug resistance (112), there are few parallels to chemoprevention. The MCF-7 cell line is derived from a pleural effusion, and the cells are, therefore, metastatic breast cancer (40). As such, the cell line does not replicate carcinogenesis in the breast or mimic primary breast cancer cells that have not developed the metastatic phenotype.

### *B. Bones*

Tamoxifen maintains bone density in the ovariectomized rat (29, 31), and these observations have been translated to clinical trials. Sporadic reports (32, 139) and placebo-controlled randomized trials (33, 140) demonstrate that tamoxifen can increase bone density in the lumbar spine, forearm, and neck of the femur by 1–2%. Although the increases are modest compared with the results obtained with estrogen use or bisphosphonates (≈ 5% increase in bone density), tamoxifen produced a significant decrease in hip and wrist fractures as a secondary end point in the breast cancer prevention trial (141). There is, however, a report that tamoxifen can reduce bone density in premenopausal women by 1–2% (142), but the decrease appears to be without clinical significance as there is no increase in the fracture rate. The reason for this may be the fact that a small decrease in bone density in a premenopausal woman is still well above the range of bone densities observed for women in their late 60s and 70s at risk for fractures.

### *C. Lipids*

Tamoxifen reduces circulating cholesterol (34, 35). Low-density lipoprotein cholesterol is reduced by about 15%, but high-density lipoprotein cholesterol is maintained. It is hypothesized that this magnitude of fall in circulating cholesterol is a good surrogate marker for protection from coronary heart disease and atherosclerosis. In this regard there is evidence that woman who have been treated with 5 yr of adjuvant tamoxifen for breast cancer have a reduced incidence of fatal myocardial infarction (143, 144). Additionally, longer treatment (5 yr) appears to be superior to shorter treatment (2 yr) in reducing the number of hospital admissions for any cardiac condition (145). Conversely, a large study in the United States of five or more years of tamoxifen for the adjuvant treatment of breast cancer found no statistically strong evidence for the protection of women from coronary heart disease (146). Nevertheless, the incidence of coronary heart disease doubled once tamoxifen treatment was stopped, and, most importantly, there was no evidence for a detrimental effect of tamoxifen, *i.e.*, tamoxifen did not increase the rate of coronary heart disease in pre- or postmenopausal women. The reasons for the disparate results probably reflect the populations studied. Breast cancer clinical trials usually require a good general health status before enrollment. Obviously, women at high risk for a second disease, coronary heart disease, would not be enrolled into a trial evaluating the efficacy of an antiestrogenic therapy in, for example, node-negative breast cancer where the prognosis, for the majority of women, would be expected to be good. Only a prospective randomized trial in a high-risk population would provide accurate data to support a claim for cardio-protection. At this point in time, there is no evidence that tamoxifen is detrimental based on current clinical evaluations, but there is no prospective clinical evidence that tamoxifen will reduce the risk of coronary heart disease.

### *D. Uterus*

It is well known that tamoxifen produces a partial agonist action in the rat uterus (93), but the histology is different than the epithelial hyperplasia noted with estradiol (147). Until the late 1980s, there was very little information about the actions of tamoxifen in the normal human uterus. However, it is now clear that a variety of endometrial changes occur in unselected populations of women (148). The most significant finding is an increase in the stromal component rather than endometrial hyperplasia (149, 150). Despite the fact that tamoxifen has been used to treat endometrial cancer, the laboratory data suggesting that tamoxifen has the potential to encourage the growth of preexisting disease harbored in the uterus (91, 92) provoked an intense investigation of the rates of detection of endometrial cancer in women using adjuvant tamoxifen treatment for breast cancer. These data have been reviewed (151), and it is clear from the recent results of the tamoxifen prevention trial (141) that tamoxifen does not cause an excess of endometrial cancer in premenopausal women but does increase risk by 3- to 4-fold in postmenopausal women. This is consistent with the fact that women harbor 4–5 times the level of endometrial cancer than is detected clinically (152). In other words, the increase in the detection of endometrial cancer from 1 per 1,000 women per year to 3 per 1,000 women per year is consistent with the known rate of occult disease. Most importantly, the stage and grade of endometrial cancer observed in women taking tamoxifen is the same as those in the general population (141, 153).

## VI. Risk Factors for Breast Cancer

If tamoxifen is an appropriate agent to test as a chemopreventive primarily because of its extensive clinical experience for the treatment of breast cancer, then the issue becomes identification of women at risk to select as a target population for recruitment to definitive clinical trials. Family history is probably the most well recognized risk factor for breast cancer, and it is now known that two forms of risk are associated with a family history of the disease. An inherited gene mutation predisposing to breast cancer is believed to account for only 5–10% of breast cancer cases (154, 155). Although infrequent, these mutations are significant since they are associated with a lifetime risk of breast cancer development of 50–80% (156, 157). At present, two predisposition genes, BRCA1, located on chromosome 17q21 (158), and BRCA2, located on chromosome 13q12–13 (159), have been identified. Both genes are inherited in an autosomal dominant fashion and are characterized by an extremely high risk of breast cancer development, which begins at a young age. Both genes also confer an increased risk of ovarian cancer development, which in BRCA1 carriers is estimated to be 10% by age 60 (160), and is lower in BRCA2 carriers. In addition, germ line mutations of the tumor suppressor gene p53, as seen in patients with the Li-Fraumeni syndrome, may account for about 1% of breast cancer cases occurring in women age 40 and younger (161, 162).

Most women with a family history of breast cancer do not have the genetically transmitted form of the disease, and therefore their increase in risk is much less than that seen in women who have inherited a predisposition gene. The cumulative probability that a 30-yr-old woman with a mother and sister with breast cancer will develop breast cancer by the age of 70 is reported to be between 7% and 18% (163, 164). While this risk increases as the number of relatives with breast cancer increases, the probability of cancer development if both a mother and sister have bilateral breast cancer has been reported to be only 25% (162, 164). The cumulative risk of breast cancer development in women with a family history of breast cancer rarely exceeds 30%, making it critically important to distinguish those women with hereditary breast cancer from those with a family history of the disease. Factors that should increase the clinician's index of suspicion that a woman is at risk for genetically transmitted breast cancer include multiple relatives (maternal or paternal) with the disease, a family history of ovarian cancer in association with breast cancer, and a family history of bilateral and/or early onset of breast cancer. Although not all women with these factors will have genetically transmitted breast cancer, a referral for genetic counseling will allow the construction of a detailed pedigree to estimate both breast cancer risk and the competing causes of death due to an increased risk of the development of other types of cancer.

Breast cancer is clearly related to endogenous hormones, and numerous studies have linked breast cancer risk to the age of menarche, menopause, and first pregnancy. Although the absolute age-specific incidence of breast cancer is higher in postmenopausal than premenopausal women (165), the absolute rate of rise of the curve is greatest up to the time of menopause, and then slows to one-sixth of that seen in the premenopausal period. Further support for the promotional role of estrogen in breast cancer development comes from the observations that early menarche (166), late menopause (167), nulliparity, and late age at first birth (168) all increase the risk of breast cancer development. An increased number of ovulatory cycles is suggested to be the common mechanism of increased risk.

Other hormonal risk factors have been suggested but are not as well established. Abortion, whether spontaneous or induced, has been reported by some authors to increase risk (169, 170), while other studies have found no relationship between abortion and breast cancer risk (171, 172). Studies of the effect of lactation on breast cancer risk have also been inconclusive (173, 174), but recent studies have suggested that a long duration of lactation reduces breast cancer risk in premenopausal women (175). Physical activity in adolescence is reported to decrease risk, perhaps due to a higher rate of anovulatory cycles (176, 177), but an increased level of physical activity later in life has not been shown to reduce breast cancer risk (178). Postmenopausal obesity has also been shown to increase risk (179), perhaps due to increased peripheral estrogen production, but this relationship between weight and risk is not observed in premenopausal women. In fact, some studies have reported an inverse relationship between weight and risk at a younger age (180).

The effects of exogenous hormones in the form of oral contraceptives and hormone replacement therapy on breast cancer risk have been studied extensively, but few firm conclusions may be drawn. Overall, there is no convincing evidence of an increase in breast cancer risk in women who have ever used oral contraceptives (181). However, some studies have suggested that the long-term use of oral contraceptives in young women before first birth may increase breast cancer risk (182, 183). Two recent meta-analyses of the effect of estrogen replacement therapy demonstrate small but statistically significant increases in risk for users (184, 185). However, Steinberg *et al.* (184) noted no increase in risk until after at least 5 yr of estrogen use, after which a proportional increase in risk for each year of estrogen use was observed, while Sillero-Arenas *et al.* (185) did not observe a significant association between duration of hormone replacement therapy and breast cancer risk. In summary, although hormonal risk factors are clearly implicated in the pathogenesis of breast cancer, most of them are associated with a RR of 3 or less of breast cancer development (Table 4), and the presence of a single hormonal risk factor is insufficient to classify a woman as high risk.

The relationship of benign breast disease to breast carcinoma was a subject of confusion for many years. The use of a standard classification of benign breast diseases as nonproliferative, proliferative, or proliferative with atypia has resolved much of the controversy. The histological diagnoses comprising these categories are shown in Table 5. Nonproliferative disease is associated with no increase in breast cancer risk, while proliferative disease increases risk by a factor of 1.5–2.0, and atypical hyperplasia by a factor of 4–5. Approximately 70% of palpable breast masses contain nonproliferative disease (186), and only 3.6% are atypical hyperplasia. The incidence of atypia is somewhat higher in biopsies performed for mammographic lesions, ranging

TABLE 4. Magnitude of known breast cancer risk factors

| RR <2 | RR 2–4 | RR >4 |
|---|---|---|
| Early menarche | Age >35 first birth | Gene mutations |
| Late menopause | First-degree relative with breast cancer | Lobular carcinoma *in situ* |
| Nulliparity | Radiation exposure | Ductal carcinoma *in situ* |
| Proliferative benign disease | Prior breast cancer | Atypical hyperplasia |
| Obesity | | |
| Alcohol use | | |
| Hormone replacement | | |

RR, Relative risk.

TABLE 5. Classification of benign breast disease

| Nonproliferative (no increase in risk) | Proliferative (RR 1.5–2.0) | Proliferative with atypia (RR 4.5–5.0) |
|---|---|---|
| Cysts, micro or macro | Papilloma | Atypical ductal hyperplasia |
| Duct ectasia | Sclerosing adenosis | Atypical lobular hyperplasia |
| Fibroadenoma | | |
| Mastitis | | |
| Fibrosis | | |
| Metaplasia, squamous or apocrine | | |
| Mild hyperplasia | | |

from 7–10% (187, 188). However, the risk of breast cancer development 15 yr after a diagnosis of atypical hyperplasia is only 8% in the absence of a family history of breast cancer. Proliferative breast disease is also noted more frequently in women with a significant family history of breast cancer than in controls, further supporting its role as a risk factor (189).

Another benign breast lesion that is clearly associated with an increased risk of breast cancer development is lobular carcinoma *in situ* (LCIS). In the past, LCIS was thought to be a malignant lesion, albeit one with a favorable prognosis. However, the finding that LCIS is associated with a risk of breast cancer development of approximately 1% per yr, the observation that the risk of carcinoma is equal in both breasts, and the finding that neither the extent of LCIS in the breast nor its presence at a margin of resection influence the risk of subsequent cancer have led LCIS to be regarded as a risk factor for breast cancer development rather than the actual precursor of carcinoma (190).

A number of environmental factors have also been linked to breast cancer risk. Exposure to ionizing radiation, whether secondary to nuclear explosion or medical procedures, has been clearly demonstrated to increase breast cancer risk (191–193). The level of risk varies with the age of exposure, with a minimal increase in risk observed for exposure in women older than 40 yr. A larger amount of attention has been directed toward the role of diet in the etiology of breast cancer. This link has been suggested by the large international variation in breast cancer incidence rates and the observation that national per capita fat consumption correlates with breast cancer incidence and mortality (194). However, prospective studies of diet and breast cancer risk have failed to identify a relationship between dietary fat intake and breast cancer incidence for up to 10 yr of follow-up (195). The lack of a relationship between dietary fat intake and cancer risk within the context of a Western diet is confirmed by a pooled analysis of seven cohort studies involving a total of 337,816 women, which demonstrated no difference in risk for women with the lowest and highest quintile of fat intake (196). However, all of these studies have addressed fat intake during adult life, and they do not exclude the possibility that fat intake during childhood and adolescence may influence subsequent breast cancer risk.

Stronger evidence exists to support an association between alcohol and breast cancer. A meta-analysis of 12 case control studies demonstrated a RR of 1.4 for each 24 g of alcohol consumed daily (197). Defining a relationship between age of alcohol consumption and breast cancer risk is more difficult, with conflicting data on the importance of drinking early in life (198, 199). A summary of the magnitude of increase in risk associated with the factors discussed is provided in Table 4.

### *A. Interactions among risk factors*

A major problem in the clinical identification of the "high risk woman" is the lack of knowledge of the interactions among the various factors known to alter breast cancer risk, since the majority of studies have focused on defining individual risk factors. Most women have a combination of factors that both increase and decrease risk, complicating the assessment of an individual's level of risk. In addition, it is unclear whether the risk conferred by multiple risk factors is additive, multiplicative, or varies with the risk factor under study.

The interactions between a family history of breast cancer and other risk factors have been examined, often with conflicting results. Dupont and Page (186) observed that the combination of atypical hyperplasia and a family history of a first-degree relative with breast cancer increased the RR of breast cancer to 11 times that of an index population, compared with a RR of 4.4 for atypia alone. However, Rosen *et al.* (200) found that the presence of a family history of breast carcinoma did not alter the level of risk after a diagnosis of LCIS, a lesion often considered part of a continuum with atypical hyperplasia. An analysis of data from the Nurses Health Study (201) found that in women with a mother or sister with breast cancer, known risk factors of age at menarche or menopause, parity, age at first birth, alcohol use, and the presence of benign breast disease did not further alter

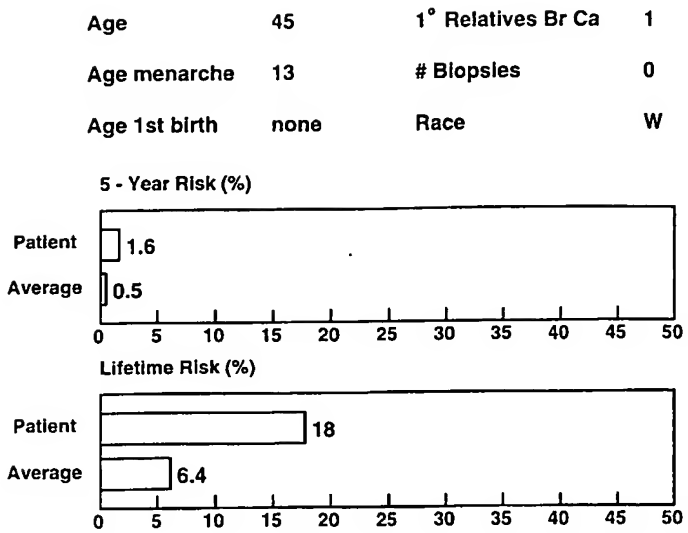


FIG. 6. Risk assessment for breast cancer in a woman with two risk factors. The woman illustrated here has a mother with breast cancer and has never had any children. The combination of these factors means that her 5-yr risk of breast cancer development is 1.6%, compared with 0.5% in a woman with no risk factors. If she lives to the age of 70, her risk will be 18% compared with 6.4% for the woman with no risk factors. This level of risk would not have qualified the woman to participate in the recently completed Breast Cancer Prevention Trial with tamoxifen.

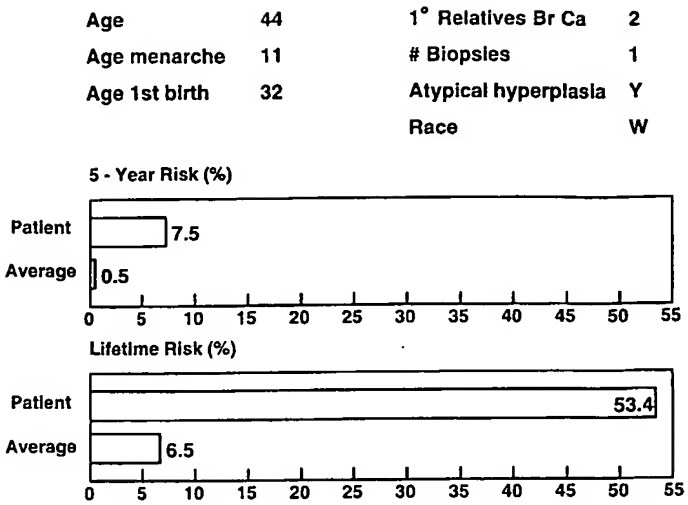


FIG. 7. Risk assessment for breast cancer in a woman with multiple risk factors. The woman illustrated here has early menarche, late age at first birth, and has a mother and sister with breast cancer. In addition, she has had a breast biopsy showing atypical hyperplasia. This combination of risk factors makes her 5-yr risk of breast cancer development 7.5% and her lifetime risk 53.4%. She is an ideal candidate to consider tamoxifen for risk reduction.

risk. In contrast, Anderson and Badzioch (202) and Brinton *et al.* (203) have reported that hormonal factors further modulate risk in women with a family history of breast cancer, although the effect varies with the factor under study.

Studies of the interaction between estrogen replacement therapy and other known breast cancer risk factors also have variable results, depending on the risk factor under study. In a meta-analysis of 16 published studies, Steinberg *et al.* (184) found that the effect of estrogen replacement did not differ among parous and nulliparous women and those with or without benign breast disease. However, an enhanced risk was observed in women with a family history of breast cancer. The analysis of the interaction among risk factors is further complicated by the fact that some factors may be important for the risk of premenopausal, but not postmenopausal, cancer and *vice versa*, and these effects may not be constant over time.

A model to predict the risk of breast cancer development in women at a given age over a defined time interval was developed by Gail *et al.* (204) using data from 4,496 matched pairs of cases and controls in the Breast Cancer Diagnosis and Demonstration Project. The model incorporates the risk factors of age at menarche, age at first live birth, number of first-degree relatives with breast cancer, and number of previous breast biopsies, and has been shown to predict risk accurately in two validation studies of women undergoing annual mammographic screening (205, 206). However, the model overpredicts breast cancer risk by 33% among women age 60 and younger who do not undergo annual screening. There are several other limitations of the model. Because only first-degree relatives are considered, it is not an appropriate model for women with extensive family histories of breast cancer, where risk may be underestimated. In women with risk due to LCIS or atypical hyperplasia, the model underestimates risk, since the highest RR for breast biopsy is 2.0. Similarly, for the woman with nonproliferative disease, the model may overestimate risk. In spite of these limitations, the model is a clinically important tool for identifying a woman's level of risk over a clinically relevant time period, after correction for competing causes of mortality. The Risk Disk, which is available from the National Cancer Institute, uses the Gail model to provide a numeric estimate of a woman's 5 yr and lifetime risk of developing breast cancer compared with an "average risk" woman of the same age. Examples are given in Figs. 6 and 7.

### *B. Identification of candidates for chemoprevention*

Women at increased risk for breast cancer would seem to be ideal candidates for chemoprevention initiatives. However, from the preceding discussion it is apparent that the problem of identification of the high-risk woman is far from solved. There is no consensus regarding what level of increase in risk is clinically relevant. The interactions among risk factors and their variability over time are poorly understood, and most of the data on risk come from studies of white women, so little is known about the impact of ethnic diversity on risk. Finally, with the exception of women with mutations of breast cancer predisposition genes, the majority of women with risk factors will not develop breast carcinoma. In addition, a recent study of the fraction of breast cancer cases in the United States due to attributable risk factors (207) found that fewer that 50% of women who develop the disease have any identifiable risk factors. A family history of breast cancer accounted for only 9.1% of cases, while relatively minor risk factors such as later age at first birth and nulliparity contributed 29.5% of cases. In a similar study, Seidman *et al.* (208) noted that only 21% of breast cancer cases in women age 30–54 and 29% of cases in women

age 55–84 occurred in women with 1 of 10 common breast cancer risk factors. The majority of women in the studies described had minor risk factors, which increased the RR of breast cancer only 2-fold, and most had only a single risk factor. This level of "increased risk" would not meet the entry criteria for the trials of breast cancer prevention in high-risk women discussed below. These data suggest that even if women with a very small increase in breast cancer risk were targeted for prevention initiatives, a large number of cases would continue to be missed.

## VII. Prevention of Breast Cancer with Tamoxifen

This section will explore progress that has been achieved in the last decade to answer the question, "Does tamoxifen have worth in the prevention of breast cancer in high risk women?". Two studies have claimed to address this question—The Royal Marsden Pilot Study (209) and the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol P-1 (141). However, the Marsden study was not designed to answer the question about the prevention of breast cancer. It was a pilot toxicology study (142) that was subsequently part of a nationwide clinical trial in Britain that planned to recruit a total of 20,000 high-risk women. The main British study is ongoing. Additionally, an Italian report of the efficacy of tamoxifen in a small number of low-risk women (~5,000) has been published (210), but again this is a small component of a 20,000-volunteer trail that has now been stopped.

### *A. Royal Marsden Pilot Study*

Powles and co-workers (211) recruited high-risk women aged 30–70 to a placebo-controlled trial using 20 mg of tamoxifen daily for up to 8 yr. Women were eligible if their risk of breast cancer was increased due to family history. Each participant had at least one first-degree relative with breast cancer under age 50, or a first-degree relative affected at any age plus an additional affected first- or second-degree relative, or a first-degree relative with bilateral breast cancer. Women with a history of benign breast biopsy and an affected first-degree relative of any age were also eligible. Women with a history of venous thrombosis, any previous malignancy, or an estimated life expectancy of fewer than 10 yr were excluded (209, 212). A total of 2,494 women consented to participate in the study, and 23 were excluded from final analysis due to the presence of preexisting ductal carcinoma *in situ* (DCIS) or invasive breast carcinoma (209) The trial was originally undertaken to evaluate the problems of accrual, acute symptomatic toxicity, compliance, and safety as a basis for subsequent large national, multicenter trials designed to test whether tamoxifen can prevent breast cancer (211). However, the trial has also been analyzed for breast cancer incidence (209). The stated goal of this pilot study was to act as a vangard for a 20,000 strong volunteer trial throughout the United Kingdom and Australia. The national study is ongoing, but the recruitment goal has been cut to 12,000.

Acute symptomatic toxicity was low for participants on tamoxifen or placebo in the pilot study, and compliance remained correspondingly high: 77% of women on tamoxifen and 82% of women on placebo remained on medication at 5 yr (212). There was a significant increase in hot flashes (34% *vs.* 20%), mostly in premenopausal women ($P < 0.005$); vaginal discharge (16% *vs.* 4%; $P < 0.005$); and menstrual irregularities (14% *vs.* 9%; $P < 0.005$), respectively. At the most recent follow-up, 320 women had discontinued tamoxifen and 176 had discontinued placebo before the study's completion ($P < 0.005$) (209).

Until their report in 1994, the Marsden group (212) observed no thromboembolic episodes; a detailed analysis of other coagulation parameters in a sequential subset of women also found no significant changes in Protein S, Protein C, or cross-linked fibrinogen degradation products. At 70 months, no significant difference in the incidence of deep vein thrombosis or pulmonary embolism was observed between groups. A significant fall in total plasma cholesterol occurred within 3 months and was sustained over 5 yr of treatment (142, 213, 214). The decrease affected low-density lipoproteins with no change in apolipoproteins A and B or high-density lipoprotein cholesterol.

In contrast, tamoxifen exerted antiestrogenic or estrogenic effects on bone density, depending on menopausal status. In premenopausal women, early findings demonstrated a small but significant ($P < 0.05$) loss of bone in both the lumbar spine and hip at 3 yr (142). It will be most important to evaluate the results at 5 and 8 yr of therapy, as current indications suggest bone stabilization rather than continued loss. In contrast, postmenopausal women had increased bone mineral density in the spine ($P < 0.005$) and hip ($P < 0.001$) compared with untreated women (142).

Finally, the Marsden group has made an extensive study of gynecological complications associated with tamoxifen treatment in healthy women. Since ovarian and uterine assessment by transvaginal ultrasound became available some time after the trial's start, many subjects did not have a baseline evaluation. Ovarian screening demonstrated a significantly increased risk ($P < 0.005$) of detecting benign ovarian cysts in premenopausal women who had received tamoxifen for more than 3 months compared with controls. There were no changes in ovarian appearance in postmenopausal women (212). A careful examination of the uterus with transvaginal ultrasonography using color Doppler imaging in women taking tamoxifen showed that the organ was usually larger; moreover, women with sonographic abnormalities had significantly thicker endometria (215). Of particular interest in this regard was the recent observation (150) that 20 mg of tamoxifen daily caused a time-dependent proliferation of the endometrium in premenopausal and early postmenopausal women. This effect appeared to be mediated by the stromal component, since no cases of cancer or even epithelial hyperplasia were observed among the tamoxifen-treated group in this Italian study with 33 women (150).

Although the Marsden pilot study has provided invaluable information about the biological effects of tamoxifen in healthy women, the trial was not designed to answer the question of whether tamoxifen prevents breast cancer. In spite of this, an analysis of breast cancer incidence was reported at a median follow-up of 70 months, when 42% of the participants had completed therapy or withdrawn (209).

During the study, 336 women receiving tamoxifen and 305 on placebo received hormone-replacement therapy. No difference in the incidence of breast cancer was observed between the groups. There were 34 carcinomas in the tamoxifen group and 36 in the placebo group—a RR of 0.98. Of the 70 cancers, only 8 were ductal carcinoma *in situ*. An analysis of the subset of women on hormone-replacement therapy did not demonstrate an interaction with tamoxifen treatment. At present there is no satisfactory answer to the question of why the Marsden Pilot study shows no decrease in breast cancer incidence in the tamoxifen arm. The authors suggest (209) that perhaps they have a high population of BRCA-1 and -2 carriers that are hormone unresponsive but this is unproven. The fact that women on the Marsden trial were allowed to take hormone replacement therapy, but the women on the NSABP P-1 trial were not is unlikely to be responsible for the Marsden result. We believe that the study, by chance, is underpowered to show a difference. If this is true, then it appears that a precise instrument to evaluate risk may be a key factor in the success of the NSABP study as well as the large volunteer population with adequate events to answer the question posed.

*B. NSABP/NCI Study*

This prospective clinical trial opened in the United States and Canada in May of 1992 with an accrual goal of 16,000 women to be recruited at 100 North American sites. The specific aim was to test the worth of tamoxifen as a preventive for breast cancer. It closed after accruing 13,388 in 1997 because of the exceptionally high-risk status of the participants. This means that the events would be adequate to establish statistical significance. The study design is illustrated in Fig. 8. Those eligible for entry included any woman over the age of 60, or women between the ages of 35 and 59 whose 5-yr risk of developing breast cancer, as predicted by the Gail model (204), was equal to that of a 60-yr-old woman. Additionally, any woman over age 35 with a diagnosis of LCIS treated by biopsy alone was eligible for entry to the study. In the absence of LCIS, the risk factors necessary to enter the study varied with age, such that a 35-yr-old woman must have had a RR of 5.07, whereas the required RR for a 45-yr-old woman was 1.79. Routine endometrial biopsies to evaluate the incidence of endometrial carcinoma in both arms of the study were also performed.

The breast cancer risk of women enrolled in the study was extremely high, with no age group having an RR of less than 4—including the over-60 group. Recruitment was also balanced, with about one-third younger than 50 yr, one-third between 50–60 yr, and one-third older than 60 yr. Secondary end points of the study included the effect of tamoxifen on the incidence of fractures and cardiovascular deaths. Most importantly, the study expects to provide the first prospective information about the role of genetic markers in the etiology of breast cancer. It will also establish whether tamoxifen has a role to play in the treatment of women who are found to carry somatic mutations in the BRCA-1 gene. (Laboratory results are not yet available.)

The first results of the NSABP study were reported in September 1998, after a mean follow-up of 47.7 months (141). There were a total of 363 invasive and noninvasive breast cancers in the participants: 124 in the tamoxifen group and 239 in the placebo group. A 49% reduction in the risk of invasive breast cancer was seen in the tamoxifen group, and a 50% reduction in the risk of noninvasive breast cancer was observed. A subset analysis of women at risk due to a diagnosis of LCIS demonstrated a 56% reduction in this group. The most dramatic reduction was seen in women at risk due to atypical hyperplasia, where risk was reduced by 86%.

The benefits of tamoxifen were observed in all age groups, with a RR of breast cancer ranging from 0.45 in women aged 60 and older to 0.49 for those in the 50–59 yr age group, and 0.56 for women aged 49 and younger (Fig. 9). A benefit for tamoxifen was also observed for women with all levels of breast cancer risk within the study, indicating that the ben-

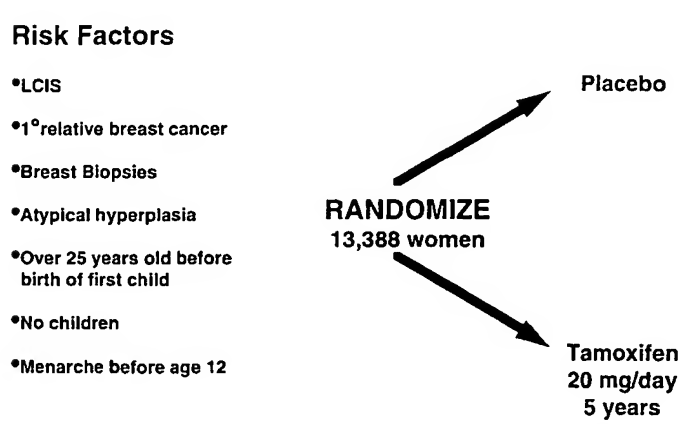


FIG. 8. The eligibility and design of the NSABP tamoxifen prevention trial. Originally the recruitment goal was 16,000 volunteers, but the actual calculated risk for the recruited group was higher than anticipated and resulted in a change in recruitment goals. A total of 13,388 women were recruited by Summer 1997, and the preliminary results were reported in April 1998. A full report was presented in September 1998 (141).

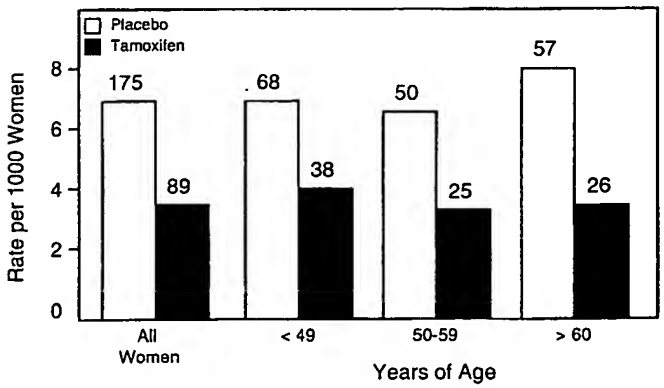


FIG. 9. The overall reduction in invasive breast cancer observed in the NSABP tamoxifen prevention trial P-1 in women at high risk for the disease, recruited to receive either tamoxifen (20 mg daily) or placebo (see Fig. 8). The women were also subdivided into age groups, and the same reduction in the incidence of breast cancer was observed. The numbers of breast cancers are shown on the *top of each histogram* for each treatment arm. [Derived from Ref. 141.]

efits of tamoxifen are not confined to a particular lower-risk or higher-risk subset. Benefits were observed in women at risk on the basis of family history and those whose risk was due to other factors.

As expected, the effect of tamoxifen was seen on the incidence of ER-positive tumors, which was reduced by 69% per year. The rate of ER-negative tumors in the tamoxifen group (1.46 per 1,000 women) did not significantly differ from that of the placebo group (1.20 per 1000 women) (Fig. 10). Tamoxifen reduced the rate of invasive cancers of all sizes, but the greatest difference between the groups was in the incidence of tumors 2.0 cm or less in size. Tamoxifen also reduced the incidence of both node-positive and node-negative breast cancer. The beneficial effects of tamoxifen were observed for each year of follow-up in the study. After year 1, the risk was reduced by 33%, and in year 5, by 69%.

Tamoxifen also reduced the overall incidence of osteoporotic fractures of the hip, spine, and radius by 19% (Fig. 11) (141). However, the overall difference approached, but did not reach, statistical significance. This reduction was greatest in women who were 50 and older at study entry. No difference in the risk of myocardial infarction, angina, coronary artery bypass grafting, or angioplasty was noted between groups (141).

This study confirmed the association between tamoxifen and endometrial carcinoma (151, 153). The RR of endometrial cancer in the tamoxifen group was 2.5. The increased risk was seen in women aged 50 and older, whose RR was 4.01. All endometrial cancers in the tamoxifen group were grade 1, and none of the women receiving tamoxifen died of endometrial cancer. There was one endometrial cancer death in the placebo group. Although there is no doubt that tamoxifen increases the risk of endometrial cancer, it is important to recognize that this increase translates to an incidence of 2.3 women per 1,000 per year who develop endometrial carcinoma.

More women in the tamoxifen group developed deep vein thrombosis than in the placebo group (141). Again, this excess risk was confined to women aged 50 and older. The RR of deep vein thrombosis in the older age group was 1.71 (95% confidence interval 0.85 to 3.58). An increase in pulmonary emboli was also seen in the older women taking tamoxifen, with a RR of approximately 3. Three deaths from pulmonary emboli occurred in the tamoxifen arm, but all were in women with significant comorbidities. An increased incidence of stroke (RR 1.75) was also seen in the tamoxifen group, but this did not reach statistical significance

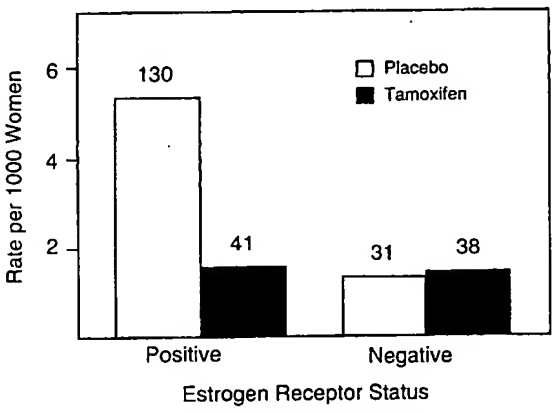


FIG. 10. The incidence of ER-positive and ER-negative breast cancer in the placebo and tamoxifen-treated arms of the NSABP tamoxifen prevention trial P-1 (see Fig. 8). The antiestrogen reduces the risk of developing ER-positive breast cancer, but there is no change in the incidence of ER-negative breast cancer. The number of breast cancers are shown on the *top of each histogram* for each treatment arm. [Adapted from B. Fisher *et al.*: *J Natl Cancer Inst* 90:1371–1388, 1998 (141). © Oxford University Press.]

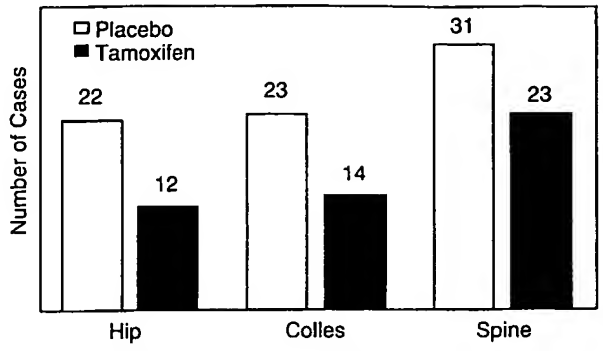


FIG. 11. The incidence of osteoporotic fractures of the hip, wrist, and spine observed in the placebo and tamoxifen-treated arms of the NSABP tamoxifen prevention trial P-1. The numbers of fractures are shown on the *top of each histogram* for each treatment arm. [Derived from Ref. 141.]

An assessment of the incidence of cataract formation was made using patient self-report. A small increase in cataracts was noted in the tamoxifen group—a rate of 24.8 women per 1,000 compared with 21.7 per 1,000 in the placebo group. There was also an increased risk of cataract surgery in the women on tamoxifen. These differences were marginally statistically significant and were observed in the older patients in the study. These findings emphasize the need to assess the patient's overall health status before making a decision to use tamoxifen for reduction of breast cancer risk.

An assessment of quality of life showed no difference in depression scores between groups. Hot flashes were noted in 81% of the women on tamoxifen compared with 69% of the placebo group, and the tamoxifen-associated hot flashes appeared to be of no greater severity than those in the placebo group. Moderately bothersome or severe vaginal discharge was reported by 29% of the women in the tamoxifen group and 13% in the placebo group. No differences in the occurrence of irregular menses, nausea, fluid retention, skin changes, or weight gain or loss were reported.

### *C. Italian study*

The third tamoxifen prevention study, performed in Italy, began in October 1992, and randomized 5,408 women aged 35 to 70 to 20 mg of tamoxifen daily for 5 yr (210). Originally, 20,000 volunteers without risk factors were to be recruited, but the study was stopped prematurely because of poor recruitment and compliance. Women were required to have had a hysterectomy for a nonneoplastic condition to obviate concerns about an increased risk of endometrial carcinoma. There was no requirement that participants be at risk for breast cancer development, and, in fact, those who underwent premenopausal oophorectomy with hysterectomy

(47%) actually had a reduced risk of breast cancer development. Women with endometriosis, cardiac disease, and deep venous thrombosis were excluded from the study. Although 5,408 women (mean age 51 yr old) were randomized into this study, 1,422 withdrew and only 149 completed 5 yr of treatment. A valid breast cancer prevention study would only be possible if more than 10,000 normal-risk women had completed 5 yr of tamoxifen *vs.* 10,000 on placebo.

The incidence of breast cancer did not differ between groups, with 19 cases in the tamoxifen group and 22 in the placebo group. Tumor characteristics, including size, grade, lymph node status, and receptor status, also did not differ between groups.

The incidence of thrombophlebitis was increased in the tamoxifen group. Fifty-six women experienced a total of 64 events: 38 women in the tamoxifen group and 18 women in the placebo group ($P = 0.0053$). However, 42 of these cases were superficial phlebitis. No differences in the incidence of cerebrovascular ischemic events were observed.

### *D. Conclusions*

Based on a single trial with a positive result and two with negative results, it may seem, at first glance, that the role of tamoxifen in breast cancer prevention remains unresolved. However, critical differences exist between these three studies (see characteristics in Table 6).

The negative finding in the Italian study (210) is readily explained by the relatively low risk of breast cancer development in the study population, the high dropout rate, the fact that the volunteers were young women, and the small number of participants who completed 5 yr of treatment. Despite these problems, the Italian study shows a trend toward statistical significance among women who took tamoxifen for more than 1 yr, suggesting that with further follow-up, the results of this study may become positive with benefit for the women who took tamoxifen. At present, the only conclusion that can be drawn from this study is that the possible benefits of tamoxifen are likely to be small in women with an average or decreased risk of breast cancer.

The Royal Marsden study was initially described as a pilot study to examine toxicity and compliance (211, 212, 214), which would serve as a feasibility assessment for a larger trial to determine whether tamoxifen prevents breast cancer. In spite of being designed as a pilot study, the trial is now said to have a 90% power to detect a 50% reduction in breast cancer incidence, yet shows no effect (209). The authors suggest that the positive results of the NSABP trial at 3.5 yr of follow-up are most likely due to the treatment of clinically occult carcinoma, rather than the prevention of new breast cancers. However, of the 363 total cancers in the NSABP study (141), 99 (28%) were DCIS, compared with 11% of the 70 cancers in the Royal Marsden study. The higher percentage of DCIS in the NSABP trial indicates that the detection of subclinical cancers occurred, and that any treated occult cancer was not truly amenable to detection by currently available means. Whether occult carcinoma was treated or true prevention occurred, a significantly greater number of women were spared surgery, irradiation, and chemotherapy. The data from the Overview Analysis (84) do not support the contention that these cancers will become clinically evident when tamoxifen is stopped, since the reduction in contralateral breast cancer persists through 10 yr even though tamoxifen treatment was stopped at 5 yr.

Overall, the results of the NSABP trial (141), with its large study population, clearly support the benefit of tamoxifen for breast cancer prevention in high-risk women. These findings are consistent with laboratory observations and with the contralateral breast cancer risk reduction seen with tamoxifen therapy (Fig. 4).

Tamoxifen was approved in 1998 for the reduction of risk in pre- and postmenopausal women with a high risk of breast cancer. The results of the NSABP prevention trial have established tamoxifen as the current standard of care and also opened the door for the evaluation of other agents, which might have an improved safety or efficacy profile in clinical trial. Tamoxifen causes rat liver carcinogenesis (216, 217), and there is an association with an increased incidence of endometrial cancer (151, 153). Although it is believed that metabolic activation of tamoxifen to a DNA adduct (218, 219) is unique to the rat liver (220–226), an agent that did not produce liver tumors in rats and was less estrogenic in the rodent uterus might have value for application as a breast cancer preventive. Raloxifene is being tested in the STAR trial (*Section IX*) against tamoxifen primarily because there is no evidence of rat liver carcinogenesis and there is less estrogen-like actions in the rodent uterus. It is possible that these laboratory qualities could translate into fewer endometrial cancers in women during raloxifene therapy, but this can only be established in large prospective clinical trials.

## VIII. Biological Basis for Raloxifene as a Breast Cancer Preventive

Raloxifene [originally named keoxifene or LY 156758 (227)] was discovered as part of the breast cancer program at

TABLE 6. A comparison of patient characteristics in the tamoxifen prevention trials

| Characteristic | NSABP | Royal Marsden | Italian |
|---|---|---|---|
| Sample size | 13,388 | 2,471 | 5,408 |
| Women years of follow-up | 46,858 | 12,355 | 5,408 |
| Participants < age 50 | 40% | 62% | 36% |
| First-degree relative with breast cancer | 55% | 55% | 18% |
| >2 First-degree relatives with breast cancer | 13% | 17% | 2.5% |
| Use of HRT | 0% | 42% | 8% |
| Breast cancer incidence/1,000 | | | |
| Placebo | 6.7 | 5.5 | 2.3 |
| Tamoxifen | 3.4 | 4.7 | 2.1 |

HRT, Hormone replacement therapy.

the laboratories of Eli Lilly & Co. in Indianapolis, IN. The drug has a high binding affinity for ER (228, 229) primarily because it has strategically located phenolic groups (see Fig. 1). However, raloxifene and an analog LY 117018 (90, 230–232) are short acting compounds because of poor bioavailability due to rapid phase II metabolism. Indeed, a concern in the early clinical trials for the treatment of breast cancer was an inability to monitor blood levels. Although numerous assays are available to monitor tamoxifen and its metabolites (105), the structure of raloxifene does not permit the use of similar chemical methods of detection. Tamoxifen is easily converted from a triphenylethylene to a phenanthrene by UV light so that fluorescence detection has been used for two decades to measure drug levels as small as 1 ng/ml serum. The analytical technique currently used to monitor raloxifene has not been published. Nevertheless, raloxifene has limited clinical experience for the treatment of breast cancer. The initial study conducted at the MD Anderson Hospital in Houston showed no responses in heavily pretreated patients with stage IV disease (233). A second small study of 18 ER-positive patients with previously untreated metastatic disease showed modest response rates of 30%, with a dose of 300 mg daily (234). The key issue, which has not yet been addressed, is cross-resistance between raloxifene and tamoxifen. The use of raloxifene for the prevention of osteoporosis after 5 yr of adjuvant tamoxifen cannot be assumed to be safe for the patient with breast cancer until this issue is resolved. A clinical trial comparing adjuvant treatment with tamoxifen for 5 yr to treatment with tamoxifen followed by raloxifene is needed to determine whether raloxifene-stimulated tumor growth is a clinical reality.

The rationale for the use of raloxifene as a breast cancer preventive is based solely on an hypothesis formulated when SERM was first recognized (25, 29).

*We have obtained valuable clinical information about this group of drugs that can be applied in other disease states. Research does not travel in straight lines, and observations in one field of science often become major discoveries in another. Important clues have been garnered about the effects of tamoxifen on bone and lipids, so it is possible that derivatives could find targeted applications to retard osteoporosis or atherosclerosis. The ubiquitous application of novel compounds to prevent diseases associated with the progressive changes after menopause may, as a side effect, significantly retard the development of breast cancer. The target population would be postmenopausal women in general, thereby avoiding the requirement to select a high-risk group to prevent breast cancer (25).*

This new strategy to prevent breast cancer was described in 1990 (25). The evidence for the application of raloxifene in this new paradigm will be presented.

### *A. Antitumor actions*

Raloxifene inhibits the growth of dimethylbenzanthracene-induced rat mammary carcinomata (235) but, dose for dose, tamoxifen is more effective. More importantly for the proposed evaluation as a preventative, raloxifene reduces the incidence of *N*-nitrosomethylurea-induced tumors (30, 236) if given after the carcinogen but before the appearance of palpable tumors (Fig. 12). However, as would be anticipated with a drug that has a short biological half-life, raloxifene is not superior to tamoxifen at equivalent doses (30). There is no doubt that raloxifene and its analogs are effective and potent inhibitors of the growth of breast cancer cells in culture (237, 238), but the complication of first-pass metabolism *in vivo* reduces potency. For this reason, doses above 60 mg raloxifene daily have been tested in clinical trial to prevent osteoporosis. As stated previously, raloxifene has only modest antitumor activity for the treatment of advanced disease (234). Only high-dose therapy (up to 300 mg daily) has been tested.

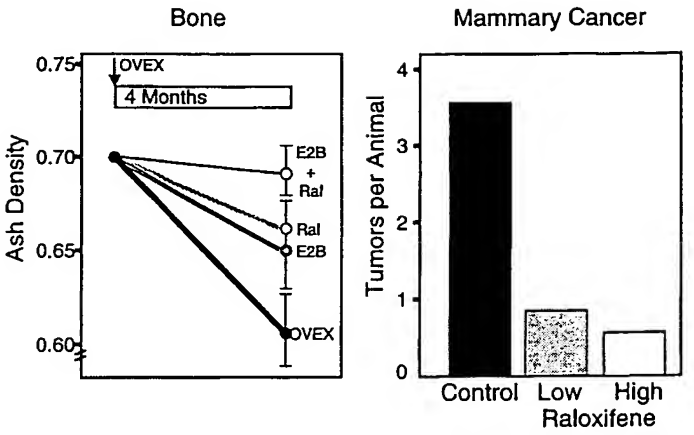


FIG. 12. A comparison of the effects of raloxifene (Ral) on femur ash density in ovariectomized (OVX) rats (29) and the incidence of rat mammary tumors after the administration of NMU. Rats were treated with 100 $\mu$g and 500 $\mu$g raloxifene daily to prevent mammary carcinogenesis (30). These data illustrate the target site specificity of a drug predicted (25) to have the potential to prevent osteoporosis and breast cancer simultaneously. $E_2B$, Estradiol benzoate, 50 $\mu$g daily orally.

Based on the hypothesis that raloxifene could reduce the incidence of breast cancer as a beneficial side effect of the prevention of osteoporosis (25), the placebo-controlled trials with raloxifene have been monitored. There are two separate data bases to test the hypothesis. First, an ongoing single trial entitled Multiple Outcomes of Raloxifene Evaluation (MORE) has randomized 7,704 postmenopausal women (mean age 66.5 yr) who had osteoporosis (hip or spine bone density at least 2.5 SD below normal mean or had vertebrate fractures) and no history of breast or endometrial cancer, to placebo, or 60 or 120 mg raloxifene daily. Results at 2 yr, with a total of 32 cases of breast cancer confirmed, indicate a 70% reduction in the risk of breast cancer (239). The second data base pools all placebo-controlled trials and includes 10,553 women monitored for, on average, 3 yr. In this group a 54% reduction in the incidence of breast cancer in the raloxifene-treated patients is observed (240, 241). As was noted in the tamoxifen study, raloxifene reduces the incidence of ER-positive breast cancer and has no effect on the incidence of ER-negative breast cancer. A comparison of the overall results with raloxifene, the NSABP prevention trial, and the small chemoprevention studies with tamoxifen is shown in Fig. 13. It should be pointed out that the data from the raloxifene study really represent three groups: one placebo control and two doses of raloxifene, 60 and 120 mg daily. Since the raloxifene data are pooled and represented in the abstracts as a percent of control, the events that can be cal-

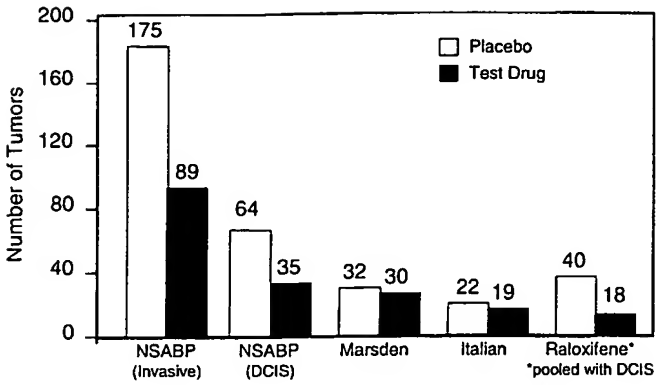


FIG. 13. A comparison of the able-to-be-evaluated events observed in the studies to reduce the incidence of breast cancer. The NSABP P-1 trial is the only prospective clinical trial designed to test the worth of an antiestrogen to prevent breast cancer in 13,388 high-risk women. The figure illustrates the effect of tamoxifen on both invasive and noninvasive (ductal carcinoma *in situ* DCIS) breast cancer. By contrast, the Royal Marsden Study is a pilot project (209) originally designed to be a toxicity evaluation (211) in 2,471 high-risk women, and the Italian study reports (210) at least one year's data from an original population of 5,408 young women of normal risk. Finally, the raloxifene data that can only be estimated from published abstracts (240, 241), constitute a secondary end point from 10,553 postmenopausal women in osteoporosis trials. The reported cases are both invasive and noninvasive breast cancers.

culated are artificially high. A more accurate representation of total events would be 30 (control), 15 (60 mg raloxifene daily), and 15 (120 mg raloxifene daily) for all reported invasive and noninvasive cancers at 3 yr. Clearly, these are small numbers compared with the NSABP study. However, the result with raloxifene is strong preliminary data as a basis for the STAR trial, which compares tamoxifen, the standard of care, with the test drug raloxifene in women with a high-risk for breast cancer (see *Section IX*).

### *B. Bones*

Raloxifene can maintain bone density in ovariectomized rats (Fig. 12) (28, 242–248). Urinary pyridinoline and serum osteocalcin are elevated after ovariectomy, but the elevations are reduced by raloxifene, thus indicating that raloxifene inhibits bone loss by reducing bone resorption (243). In general, raloxifene does not maintain bone density in the ovariectomized rat to the level observed in an intact animal (242), but efficacy appears equivalent to estrogen treatment although without increases in uterine weight (242).

Preliminary studies in 251 normal postmenopausal women randomized to placebo, raloxifene (200 mg daily), raloxifene (600 mg daily), or Premarin (Wyeth-Ayerst Laboratories, Inc., Philadelphia, PA; 0.625 mg daily) show decreases in serum alkaline phosphatase, serum osteocalcin, urinary pyridinoline, and urinary calcium excretion with raloxifene that was no different than estrogen (249). However, the doses of raloxifene are far higher than the 60 mg/day currently recommended for the prevention of osteoporosis. Evaluation of raloxifene, 60 mg/day, on bone remodeling in early postmenopausal woman, using calcium tracer kinetic methods, found that although remodeling suppression was greater for estrogen, the remodeling balance was the same for the two agents (250). The authors concluded that raloxifene acted on bone as an estrogen agonist. Indeed, these results are consistent with the finding that raloxifene increases bone density by 2.4 ± 0.4% in the lumber spine and 2.4 ± 0.4% for the total hip (251). Although the percent increases in bone density are not as high as would be anticipated with estrogen or bisphosphonates, it is now clear that raloxifene produces a 40% decrease in spine fractures. There are, however, no reports of a significant decrease in hip fractures with raloxifene. This contrasts with the 50% decrease noted with tamoxifen in the prevention study (141) and adds further support for the need to compare and contrast the clinical endocrinology of tamoxifen and raloxifene in the STAR trial (see *Section IX*).

### *C. Lipids*

Raloxifene produces a significant decrease in low-density lipoprotein cholesterol, but high-density lipoprotein cholesterol remains the same (249, 251, 252). Additionally, triglycerides do not rise during raloxifene treatment. Laboratory data from the rabbit (253) strongly support the value of raloxifene to prevent atherosclerosis. However, data from primates fed high-cholesterol diets do not show a benefit for raloxifene (254). These results have proved to be controversial (255), as both hormone replacement therapy and tamoxifen show positive results in the primate model. To address the issue directly, a prospective randomized clinical trial is in place to address the question of whether raloxifene has merit for the reduction of risk for coronary heart disease in postmenopausal women with elevated risk factors. The study, referred to as Raloxifene Use for The Heart (RUTH), will randomize 10,000 high-risk women to placebo or raloxifene (60 mg daily) treatment for 5 yr. Results should be available by 2005.

### *D. Uterus*

Raloxifene and its analogs have low estrogen-like actions in the rat uterus (227, 229–231). Indeed, the raloxifene analog LY117018 is able to block (at high doses) the estrogen-like effect of tamoxifen on the rat uterus (231). However, raloxifene and its analogs cannot be classified as pure antiestrogens in these tests. There is not a complete lack of uterotropic properties (256, 257), and estrogen-regulated genes, such as the PgR, are partially activated (232). Also, mechanistically raloxifene cannot be described as a pure antiestrogen (117).

Raloxifene is receiving a rigorous evaluation in the human uterus. This is important because the drug is used to prevent osteoporosis. A current evaluation in women screened to ensure the absence of preexisting endometrial abnormalities shows that raloxifene, unlike estrogen, does not increase endometrial thickness (258). Although there is not enough information to prove that raloxifene reduces the incidence of endometrial cancer in treated women, there is no evidence to suggest that raloxifene increases the risk for endometrial cancer. Raloxifene does have less estrogenicity in the uterus than tamoxifen, and it only increases the growth of human endometrial carcinomas under laboratory conditions by about 50% of that noted with tamoxifen (259). This, coupled

with the preliminary data with raloxifene as a potential preventive for breast cancer in elderly woman (239–241), is sufficient to propose testing against the current standard of care, tamoxifen.

### IX. Study of Tamoxifen And Raloxifene (STAR)

The STAR trial is a phase III, double-blind trial that will assign eligible postmenopausal women to either daily tamoxifen (20 mg orally) or raloxifene (60 mg orally) therapy for 5 yr. Trial participants will also complete a minimum of two additional years of follow-up after therapy is stopped.

The STAR trial's primary aim is to determine whether long-term raloxifene therapy is effective in preventing the occurrence of invasive breast cancer in postmenopausal women who are identified as being at high risk for the disease. The comparison is to be made to the established drug, tamoxifen. Its secondary aim is to establish the net effect of raloxifene therapy, by a comparison of cardiovascular data, fracture data, and general toxicities with tamoxifen. It is clear that the activation or suppression of various target sites around a woman's body is similar for tamoxifen and raloxifene, but an evaluation of the overall comparative benefits of the agents will be an important new clinical data base for raloxifene in postmenopausal women.

Premenopausal women at risk for breast cancer are, currently, not eligible for the STAR trial. Although there is extensive information about the efficacy of tamoxifen in premenopausal breast cancer patients (84) and women at risk for breast cancer (141), clinical experience with raloxifene is confined to monitoring the action of the drug in postmenopausal women. Raloxifene is classified as an antiestrogen with less estrogen-like actions than tamoxifen (229–231). However, tamoxifen has been shown to produce a small decrease in bone density in premenopausal women (142), and there is concern that raloxifene might produce greater decreases in bone density. The National Cancer Institute is currently conducting a randomized study of raloxifene (60 mg daily and 300 mg daily) in high-risk premenopausal women to address the issue of raloxifene and bone density. Additionally, short-term raloxifene treatment (5 days or 28 days) causes elevations in circulating estradiol but does not prevent ovulation (260), consistent with the known elevation of steroid hormones produced by tamoxifen in premenopausal breast cancer patients (261). The changes in endocrine function produced by raloxifene will also be assessed as a prelude to the recruitment of premenopausal high-risk women to the STAR trial.

The results from the STAR are anticipated by 2006. Clearly it will be invaluable to establish the overall benefits of the drugs with regard to breast cancer incidence, coronary heart disease, and osteoporosis. The comparisons of endometrial cancer will be most instructive because the standard of care, *i.e.*, self-reporting, will be employed in the STAR trial rather than routine screening with annual biopsies.

### X. The Future of Prevention

The concept of the chemoprevention of cancer was proposed in the mid-1970s by Sporn and colleagues (262, 263). Essentially, effective implementation of the strategy requires a target to prevent either the initiation or the promotion of the cancer cell (or both). The key to success is, therefore, a well defined target so that a selective action can be applied without general toxicity. The idea that breast cancer could be prevented was first proposed by Lacassagne (7) in 1936. He suggested that an antagonist to estrogen action could prevent the disease. The target became the ER (22), but serendipitously the antiestrogens tamoxifen and raloxifene, which block breast cancer cell growth, were also found to modulate the physiological requirements for estrogen action selectively at other target sites (28–30).

The major clinical question for the current application of tamoxifen as a chemopreventive is when should the 5-yr course be taken and how long will the effects last to protect a woman at elevated risk for breast cancer? The simple answer to the first part of the question is that a woman who is found to fit the elevated risk criteria for breast cancer will receive benefit through a 55% risk reduction whenever she takes tamoxifen. However, since there are no rules that can define when a woman will develop breast cancer if she is found to be at risk, then earlier rather than later would seem to be the appropriate strategy. The answer to the duration of benefit part of the question is less clear, but there are clues that indicate that 5 yr of tamoxifen therapy results in protection, *i.e.*, risk reduction, for at least 5 yr after the drug is stopped, based on the data about contralateral breast cancer from the Overview Analysis (84). At this time, further follow-up is not available. Clearly, it will be important to discover the mechanism for the long-term beneficial effects of tamoxifen as a chemopreventive, as this could be exploited further. Similar questions about the optimal duration of raloxifene therapy for prevention will need to be addressed in the future. In the case of raloxifene, this is not as important an issue because long-term therapy for the prevention of osteoporosis is necessary.

Finally, there is the issue of whether 5 yr of tamoxifen will be sufficient for prolonged protection from breast cancer, or whether longer durations of initial treatment will provide longer periods of protection. For example, after 10 or 15 yr of treatment can a woman get 20 yr of risk reduction? However, there is a reluctance to consider this type of clinical experiment at present because of a concern about the development of tamoxifen-stimulated primary breast cancer. The concern comes from the literature about breast cancer treatment. Although 10 yr of adjuvant tamoxifen appears to be less effective than 5 yr for the treatment of breast cancer (264), this is not useful evidence to support the restricted application of tamoxifen as a chemopreventive. Drug resistance by metastatic breast cancer cells can probably develop much more rapidly than can occur during the process of carcinogenesis for primary breast cancer. As a result, it may be important to evaluate longer durations of tamoxifen as a chemopreventive agent in clinical trial. Be that as it may, the challenge for the present is first to establish the efficacy of raloxifene compared with tamoxifen in the STAR trial, and then to determine the optimal application of SERMs as a new drug group for the benefit of women's health.

Finally, a new generation of agents that are specifically designed to modulate ER$\alpha$ and -$\beta$ selectively will become

available for clinical testing within the next decade. A number of postmenopausal diseases will be targeted, such as osteoporosis and coronary heart disease, but the beneficial side effects should include a reduction in uterine and breast cancer in the general population. The present reduction of breast cancer in high-risk women by 50% is an important first step that has resulted from the rational application of translational research. The challenge for the future is to apply developing laboratory knowledge about the mechanisms of carcinogenesis to prevent breast cancer completely.

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